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The characteristics and clinical significance of mucin levels in bronchoalveolar lavage fluid of patients with interstitial lung disease

Li Wei,[1,2] Jing Zhao,[1,3] Jing Bao,[1] Yanliang Ma,[1] Ying Shang,[1] Zhancheng Gao[1]

[1]Department of Respiratory and Critical Care Medicine, Peking University People's Hospital, Beijing, China
[2]Department of Respiratory Medicine, Affiliated Hospital of Taishan Medical University, Taian, Shandong, China
[3]Department of Respiratory Medicine, Dushanzi People's Hospital, Karamay, Xinjiang, China

Correspondence to
Dr Yanliang Ma, Department of Respiratory and Critical Care Medicine, Peking University People's Hospital, Beijing 100044, China; helloyl1979@126.com

LW and JZ contributed equally.

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ABSTRACT

To investigate the expression and clinical significance of secretory mucins in patients with interstitial lung disease (ILD). The bronchoalveolar lavage fluid (BALF) concentrations of mucins (MUCs) from 27 patients with ILD, 6 patients with lung cancer, 8 patients with pleural effusion and 9 patients with bronchiectasis were determined by ELISA. The concentration of MUC5AC was significantly increased in patients with ILD (12.84±15.02 ng/mL) compared with patients with pleural effusion (4.33±2.51 ng/mL), lung cancer (8.02±5.57 ng/mL) or bronchiectasis (6.08±2.40 ng/mL) ($p<0.01$). The MUC2 level (10.23±9.27 ng/mL) was significantly elevated in patients with ILD than in those with pleural effusion (6.21±3.28 ng/mL) or bronchiectasis (5.73±1.51 ng/mL) (both $p<0.05$). Patients with ILD (104.64±61.61 ng/mL), lung cancer (148.45±169.24 ng/mL) or bronchiectasis (123.68±63.28 ng/mL) had significantly greater IL-8 levels than in those with pleural effusion (76.46±2.16 ng/mL) ($p<0.05$). A significant positive correlation was detected between the MUC5AC concentration and the lymphocyte percentage in BALF of patients with ILD ($r=0.504$, $p=0.007$). Lung function tests of patients with ILD exhibited various degrees of restrictive ventilation dysfunction and reduced diffusing capacity. The MUC5AC levels in BALF were negatively correlated with forced expiratory volume in 1 second (FEV_1)/forced vital capacity ($r=-0.761$, $p=0.000$), FEV_1 predicted value (FEV_1/pred) ($r=-0.668$, $p=0.002$), and diffusing capacity ($r=-0.606$, $p=0.006$). Secretory mucins MUC5AC, MUC2 and IL-8 were highly expressed in ILD. MUC5AC level was closely correlated with the amount of inflammatory cells in BALF and the lung function parameters.

Significance of this study

What is already known about this subject?

- The expression of mucin (MUC)1 family member KL-6 protein was related to the diagnosis and prognosis of interstitial lung disease (ILD).
- Single nucleotide polymorphisms in the coding regions of MUC2 and MUC5AC were associated with familial ILD and idiopathic pulmonary fibrosis (IPF).
- IPF with honeycomb changes had metaplasia in mucous cells around the terminal bronchioles and highly expressed MUC5B.

What are the new findings?

- MUC5AC and MUC2 concentrations in bronchoalveolar lavage fluid (BALF) of patients with ILD were significantly higher compared with patients with pleural effusion.
- The MUC5AC level was positively correlated with the percentage of lymphocytes in BALF.
- The MUC5AC level was negatively correlated with the ventilation and diffusing capacity.

How might these results change the focus of research or clinical practice?

- Mucus hypersecretion exists in patients with ILD and MUC5AC may be involved in the airway inflammatory response of ILD.

INTRODUCTION

Mucins (MUCs) are macromolecular glycoproteins secreted by epithelial goblet cells and mucous cells of submucosal glands. They are important components of airway mucus. A total of 21 MUC genes have been identified so far, which can be classified into membrane-associated and gel-forming MUCs according to their characteristics.[1] In lung tissues, membrane-associated MUCs MUC1, MUC4, and MUC16 provide structural support and participate in signal transduction for cilia and extracellular matrix. Gel-forming MUCs MUC2, MUC5AC, MUC5B, and MUC19, secreted by goblet cells and mucous cells of submucosal glands, are major components of airway mucus. They determine the viscosity of the mucus.[2]

In 1985, Kohno *et al* discovered that the expression of MUC1 family member KL-6 protein reflected the impairment and regeneration of type II alveolar epithelial cells and interstitium.[3] Increasing evidence confirmed that KL-6 was associated with interstitial lung disease (ILD) such as idiopathic pulmonary fibrosis (IPF) and sarcoidosis.[2] Detection of serum or brochoalveolar lavage fluid (BALF) levels of KL-6 facilitated early diagnosis of various ILDs. KL-6 level was associated with prognosis of IPF.[4–6] These findings implied

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Table 1 Clinical characteristics of the study population

Variable	ILD	Pleural effusion	Bronchiectasis	Lung cancer
Total number	27	8	9	6
Male/female	11/16	6/2	6/3	4/2
Age (years)	66.86±12.83	45.88±17.37	58.33±12.64	69.17±8.01
WBC (10^9/L)	7.02 (5.83–9.37)	4.96±1.07	8.3±3.13	6.635 (6.035–10.085)
LY (%)	20.96±10.71	28.81±7.47	26.94±8.51	19.48±9.91
N (%)	68.26±13.05	57.62±7.49	57.72±15.14	66.08 (61.80–77.86)
Hb	124.11±14.06	122.86±5.89	118.89±9.83	106.8±7.28
ESR (mm)	45 (23–74.5)	16.75±5.25	14 (7.5–58)	44.5±44.85
FEV_1/pred (%)	82.74±25.71*	103.2±6.90	59.52±25.15	85.88±10.88
FEV_1/FVC (%)	83.78 (80.33–86.68)*	80.60±7.01	60.28±19.52	71.83±8.22
TLC/pred (%)	82.49±21.37*	97.73±6.83	102.1 (85.1–164.9)	88.15±16.43
RV/pred (%)	100.72±40.92*	100.67±12.51	164.10±87.70	99.60±12.30
RV/TLC (%)	46.08 (40.52–52.73)*	35.34±3.94	51.70±18.86	46.09±5.27
DLCO/pred (%)	51.64±22.04*	84.57±3.20	70.08±26.43	63.2±4.24

Data were expressed as mean±SD or median (range) as appropriate.
*Statistical analysis performed in 19 patients with ILD.
DLCO, diffusing capacity of the lungs for carbon monoxide; ESR, erythrocyte sedimentation rate; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; Hb, hemoglobin; ILD, interstitial lung disease; LY, lymphocyte; N, neutrophil; pred, predicted value; RV, residual volume; TLC, total lung capacity; WBC, white blood cell.

an involvement of MUC in the pathogenesis and development of ILD.

Seibold[7] *et al* found that single nucleotide polymorphisms in the coding regions of MUC2 and MUC5AC were associated with familial ILD and IPF through a full-length sequence scanning of the P-terminal portion of chromosome 11. Plantier *et al*[8] found that 53.5% of IPF with honeycomb changes had metaplasia in mucous cells around the terminal bronchioles and highly expressed MUC5B, suggesting that gel-forming MUCs may also be involved in the pathogenesis of ILD. This study intended to further explore the unique characteristics of airway MUC secretion and its relationship with the clinical features in patients with ILD.

MATERIALS AND METHODS

Study design and ethics statement

This was a retrospective study. We identified patients who met the diagnostic criteria from the database in the laboratory of Department of Respiratory and Critical Care Medicine in People's hospital, Peking University while collecting remaining BALF of cell counts examination after routine bronchoscopy for clinical need from hospitalized patients. All the patients signed informed consent on subsequent storage and use for scientific research before bronchoscopy. Clinical data were extracted from the hospitalization record.

Participants

Twenty-seven patients diagnosed with ILD who were admitted to the Department of Respiratory Medicine and Department of Rheumatology and Immunology of Peking University People's Hospital from March 2011 to December 2012 were studied. Another twenty-three patients with bronchiectasis, pleural effusions or lung cancer, diagnosed during the same period, served as controls. No patient was included in other clinical study.

Interstitial lung disease

All patients with ILD underwent high-resolution chest CT examination. Interlobular septal thickening, ground-glass opacities, reticular opacities, and consolidations were observed in both lungs.[9] Secondary causes were evaluated by reviewing medical histories and performing physical examination, laboratory tests, lung function test and bronchoscopy (including BALF analysis and transbronchoscopic lung biopsy if necessary). Eventually, 15 cases were diagnosed with connective tissue disease related ILD (CTD-ILD) (secondary to mixed CTD in five cases, Sjogren's syndrome in four cases, rheumatoid arthritis in three cases, systemic lupus erythematosus in two cases, and dermatomyositis in one case) and 5 cases were vasculitis-related ILD. Three patients met the diagnosis criteria for IPF,[10] two of whom were hospitalized for acute exacerbation. Four patients with idiopathic interstitial pneumonia who did not meet the diagnostic criteria for IPF were not classified further.

Bronchiectasis

Nine cases of bronchiectasis were diagnosed through previous medical history, clinical manifestations, signs, laboratory tests, and thoracic hgh resolution computed tomography (HRCT) findings. Traction bronchiectasis due to interstitial fibrosis was excluded.[11]

Pleural effusion

Among eight patients with pleural effusion, six cases were tuberculous pleural effusion and two were parapneumonic pleural effusion. One patient with tuberculous pleural effusion was diagnosed by thoracoscopic biopsy. All other patients were confirmed by clinical symptoms, signs, biochemistry and pathology results of pleural fluid, biochemistry, and adenosine deaminase test. No patient had interstitial lesions on chest CT.

Table 2 Total and differential cell counts in BALF

Disease	Total cell count (10^6/L)	Macrophage (%)	Lymphocyte (%)	Neutrophil (%)
Pleural effusion	0.39±0.16	91.33±3.733	7.67±3.17	0.67±0.75
ILD	0.38±0.21	61.90±24.28*	26.10±20.65*	9.93±14.04*
Lung cancer	0.43±0.28	70.07±35.38	16.00±13.16	13.86±34.69
Bronchiectasis	0.39±0.31	62.88±26.79†	12.19±3.87†	24.75±28.09†

BALF, bronchoalveolar lavage fluid; ILD, interstitial lung disease.
*p<0.01 compared with patients with pleural effusion.
†p<0.05 compared with patients with pleural effusion.

Lung cancer

Six cases of lung cancer, including four cases of lung adenocarcinoma, one case of small cell lung cancer and one case of squamous cell carcinoma were all pathologically confirmed. No features of interstitial lesions on chest CT were detected.

Measurement

Bronchoalveolar lavage fluid

All patients received bronchoscopy and bronchoalveolar lavage (BAL) for clinical need. For patients with ILD, bronchoscopy and BALF cell count was used to diagnose the underlying cause and guide the use of corticosteroids. For patients with bronchiectasis, BALF was used for pathogen detection. In the case of patients with pleural effusion, bronchoscopy was applied to eliminate tracheobronchial tuberculosis which was common in China. For patients with lung cancer, bronchoscopy was also critical for diagnosis and BALF was sent for pathological analysis. All patients signed informed consent for bronchoscopy.

BAL was performed by routine bronchoscopy under local anesthesia according to protocol.[12] The right middle lobe or left lingual lobe was selected for BAL if diffuse lesions were exhibited in the thoracic CT images, and lesions were directly lavaged for those with localized lesions on chest CT scan.

BALF was filtered through a double-layer gauze and the cell number was counted with Wright's stain.[12] If the patients signed informed consent for subsequent storage and use for scientific research before bronchoscopy, the remaining lavage fluid after clinical use would be collected. The fluid was centrifuged at 1200 r/min for 10 min and the supernatant was extracted and stored at −20°C for storage.

Double-antibody sandwich ELISA was used to measure the levels of MUC5AC (USCN LIFE SCIENCE, Houston, Texas, USA, lot number E90756Hu), MUC5B(USCN LIFE SCIENCE, Houston, Texas, USA, lot number E90684Hu), MUC2 (USCN LIFE SCIENCE, Houston, Texas, USA, lot number E90705Hu) and IL-8 (USCN LIFE SCIENCE, Houston, Texas, USA, lot number E90080Hu) in the BALF supernatant.

Lung function test

All patients underwent pulmonary function tests for clinical use except eight patients with critical conditions. Routine pulmonary ventilation and diffusing capacity were measured by Jaeger MasterLab (Erich Jaeger GmbH & CoKG, Würzburg, Germany). The technical parameters met quality standard of the European Respiratory Society and the American Thoracic Society (ERS/ATS). The operation obeyed the ERS/ATS guidelines for pulmonary function testing.[13]

Statistical analysis

SPSS V.20.0 software was used for statistical analysis. Normally distributed data were expressed as mean±SD and non-normally distributed data were represented as the median with IQR. Data among groups were compared using one-way analysis of variance with least significant difference tests or Kruskal-Wallis tests. Correlations were analyzed by bivariate Pearson's correlation. P<0.05 was considered statistically significant.

Table 3 Total and differential cell counts of BALF of the subgroup patients with ILD

Disease	Total cell count (10^6/L)	Macrophage (%)	Lymphocyte (%)	Neutrophil (%)
CTD-ILD (n=15)	0.42±0.27	61.67±24.52	25.53±19.29	11.93±17.76
Vasculitis-related ILD (n=5)	0.34±0.18	86.00 (48.5–91.75)	11.50 (4.75–38)	2.5±2.06
IPF-1 (AE)	0.16	67	26	7
IPF-2	0.3	93	2.5	4.5
IPF-3 (AE)	0.63	29	26	41
Unidentified IIP-1	0.33	28.5	27	43
Unidentified IIP-2	0.32	38	61.5	0.5
Unidentified IIP-3	0.19	17	72.5	10.5
Unidentified IIP-4	0.4	84	10	5.5

AE, acute exacerbation; BALF, bronchoalveolar lavage fluid; CTD, connective tissue disease; IIP, idiopathic interstitial pneumonia.; ILD, interstitial lung disease; IPF, interstitial pulmonary fibrosis.

Table 4 Concentrations of MUC and IL-8 in BALF

Disease	MUC5AC (ng/mL)	MUC5B* (ng/mL)	MUC2 (ng/mL)	IL-8 (ng/mL)
Pleural effusion	4.33±2.51	0.10±0.03	6.21±3.28	76.46±2.16
ILD	12.84±15.02†	0.33±1.04	10.23±9.27‡	104.64±61.61‡
Lung cancer	8.02±5.57‡	0.50±0.86	29.51±54.28‡	148.45±169.24
Bronchiectasis	6.08±2.40	0.09±0.003	5.73±1.51	123.68±63.28

*The MUC5B level in BALF of most patients was beyond the detection range of the detection kit (0.625 ng/mL~40 ng/mL).
†p<0.01 compared with patients with pleural effusion.
‡p<0.05 compared with patients with pleural effusion.
BALF, bronchoalveolar lavage fluid; ILD, interstitial lung disease; MUC, mucin.

RESULTS

Baseline characteristics

The average age of the study population was 62.08±14.76 years with a range of 21–83 years. The clinical characteristics are shown in table 1.

Total and differential cell counts of BALF

There was no significant difference in cell counts of BALF among the groups (p>0.05). The percentages of lymphocytes and neutrophils in BALF of patients with ILD or bronchiectasis were significantly higher than in those with pleural effusion (table 2). The total and differential cell counts in patients with ILD with various causes are shown in table 3. Statistical analysis was not performed due to the limited sample size.

Concentrations of MUC and interleukin (IL)-8 in BALF

Patients with ILD had a significantly higher MUC5AC concentration in BALF (12.84±15.02 ng/mL) compared with patients with pleural effusion (4.33±2.51 ng/mL), lung cancer (8.02±5.57 ng/mL) or bronchiectasis (6.08±2.40 ng/mL) (p<0.01). The BALF concentration of MUC2 for patients with ILD (10.23±9.27 ng/mL) was significantly greater than that of patients with pleural effusion (6.21±3.28 ng/mL) or bronchiectasis (5.73±1.51 ng/mL) (p<0.05). Patients with ILD, lung cancer, and bronchiectasis had significantly elevated BALF levels of IL-8 compared with patients with pleural effusion (104.64±61.61 ng/mL, 148.45±169.24 ng/mL, and 123.68±63.28 ng/mL vs 76.46±2.16 ng/mL, p<0.05). The MUC5B level in BALF of most patients was beyond the detection range of the detection kit (0.625 ng/mL~40 ng/mL), so statistical analysis was not performed (table 4). The levels of secretory MUCs in patients with different types of ILD are shown in table 5.

Correlation between MUC levels and percentage of cells in BALF

In patients with ILD, the MUC5AC concentration in BALF was positively correlated with the percentage of lymphocytes (r=0.504, p=0.007), and was negatively correlated with the percentage of phagocytes (r=−0.484, p=0.011) (table 6).

Correlation between MUC levels in BALF and lung function

A great extent of restrictive ventilator impairment with decreased diffusing capacity was observed in patients with ILD. MUC5AC levels in BALF were negatively correlated with FEV_1/FVC and FEV_1/pred. The concentration of MUC5AC in BALF was negatively correlated with diffusing capacity. There was no significant correlation between MUC2 levels and parameters of lung function test (table 6).

DISCUSSION

Mucus hypersecretion is an important characteristic of respiratory diseases such as bronchial asthma, chronic obstructive pulmonary disease, and cystic fibrosis. Airway mucus secretion has unique features. Airway MUC5AC and MUC5B levels in patients with asthma were significantly higher than those in normal controls.[14] In patients with

Table 5 Concentrations of MUC and IL-8 in BALF of the subgroup of patients with ILD

Subgroup	MUC5AC (ng/mL)	MUC5B* (ng/mL)	MUC2 (ng/mL)	IL-8 (ng/mL)
CTD-ILD (n=15)	9.94 (6.46)	0.09±0.01	5.96 (4.95)	82.51 (16.11)
Vasculitis-related ILD (n=5)	9.13±3.49	0.09±0.00	10.67±8.58	79.66±6.78
IPF-1 (AE)	57.03	0.15	2.80	197.79
IPF-2	7.06	0.09	31.27	78.77
IPF-3 (AE)	13.82	0.09	8.09	107.41
Unidentified IIP-1	10.24	0.09	3.22	81.76
Unidentified IIP-2	11.83	0.08	9.39	76.71
Unidentified IIP-3	76.99	0.14	16.71	129.49
Unidentified IIP-4	10.83	0.08	4.25	83.35

Data are expressed as mean±SD or median (range).
*The MUC5B level in BALF of most patients was beyond the detection range of the detection kit(0.625 ng/mL~40 ng/mL).
AE, acute exacerbation; BALF, bronchoalveolar lavage fluid; CTD, connective tissue disease; IIP, idiopathic interstitial pneumonia.; ILD, interstitial lung disease; IPF, interstitial pulmonary fibrosis; MUC, mucin.

Table 6 Correlations between mucin levels, percentage of cells, IL-8 levels in BALF and lung function in patients with interstitial lung disease

Variable	Macrophage (%)		Lymphocyte (%)		Neutrophil (%)		IL-8		FEV_1/pred		FEV_1/FVC		DLCO		TLC	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
MUC5AC	−0.484	0.011	0.504	0.007	0.126	0.531	0.352	0.072	−0.668	0.002	−0.761	0.000	−0.606	0.006	−0.043	0.867
MUC2	0.025	0.903	0.315	0.282	−0.312	0.114	−0.243	0.223	0.282	0.243	0.041	0.868	0.138	0.573	0.264	0.290

BALF, bronchoalveolar lavage fluid; DLCO, diffusing capacity of the lungs for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; MUC, mucin; pred, predicted value; TLC, total lung capacity.

chronic obstructive pulmonary disease, airway MUC5B was significantly increased while MUC2 was less expressed.[15] However, little research has reported the effect of MUCs in ILD. Previous studies have focused on the MUC1-like glycoprotein KL-6[16] and less attention has been paid to the relationship between airway secretory MUC and ILD. Recent studies found that ILD involved pulmonary interstitium and affected alveolar lumen, peripheral airways, blood vessels and the corresponding epithelial and endothelial cells.[17] Mucus hypersecretion existed in patients with ILD, especially IPF. In the current study, MUC5AC and MUC2 concentrations in BALF of patients with ILD were significantly higher compared with patients with pleural effusion, suggesting an abnormal expression of MUC5AC and MUC2 in airways of patients with ILD.

MUC5AC and MUC2 are both expressed by goblet cells in the airway epithelium. Goblet cells are mainly distributed in the trachea, bronchi, and bronchioles and are rarely seen in small airways with a diameter of less than 2 mm. The goblet cell number in an healthy individual's airway is low. However, the goblet cell number could increase up to 30 times in diseases or conditions such as chronic airway inflammation caused by smoking, infection and oxidative stress.[18] In this study, patients with ILD had significantly increased concentrations of MUC5AC and MUC2 in the BALF. In addition, the MUC5AC level was positively correlated with the percentage of lymphocytes in BALF. Considering that the increase in lymphocytes in BALF was associated with pulmonary inflammation response,[19] our findings implied that MUC5AC and hypersecretion of goblet cells are involved in the airway inflammatory response of ILD. Pulmonary function has important clinical significance for the diagnosis and management of ILD. ILD often leads to obvious impaired restrictive ventilation and reduced diffusing capacity. In ILD, MUC5AC concentration in BALF was negatively correlated to ventilation and diffusing capacity, suggesting that MUC5AC was associated with lung function impairment. Further studies are needed to understand whether MUC5AC is associated with the prognosis of patients with ILD.

Plantier *et al* and Seibold *et al*[7 8] also observed a significantly elevated transcription level of MUC5B in peripheral airways of patients with ILD, especially for IPF. However, in the current study, the MUC5B level in BALF of patients with ILD was very low and beyond the detection range of the detection kit, which might be attributed to the different diseases' spectrum. In the current study, only three patients with IPF were included, two of which had an increased percentage of BALF lymphocytes with an admission due to acute exacerbation. BALF lymphocytes were not significantly increased in 5 patients with vasculitis-related ILD while increased lymphocytes were observed in 15 patients with CTD-ILD and 4 patients with unclassified ILD. All these evidences suggested that the airway inflammation in most of our study subjects differed from the typical neutrophil-mediated inflammatory response as exhibited in patients with IPF. This phenomenon also suggested that patients with different types of ILDs might have distinctive features of MUC secretion and further studies with larger sample sizes are necessary. A previous study[20] showed that MUC5B may play an important role in the autoregulation, pathogenesis and mucosal immune function in the

respiratory tract. But we cannot conclude the same with regard to the low level of MUC5B in our study.

ILD is a heterogeneous group of lung disorders with poor prognosis. At present, corticosteroids and immunosuppressants have been widely used for the treatment of ILD. However, side effects of hormones and immunosuppressants are commonly seen and some patients did not respond well to these drugs. A deeper understanding of the pathogenesis of ILD facilitates the discovery of new therapeutic approaches. We revealed a hypersecretion of MUC5AC and MUC2 in patients with ILD. Moreover, in patients with ILD, the Muc5AC level was positively correlated with the percentage of lymphocytes in BALF and negatively correlated with pulmonary ventilation and diffusion. However, further studies are necessary to elucidate the underlying mechanisms of hypersecretion, related influencing factors, signal transduction pathways, relationships between MUCs and airway inflammation, and the characteristics of MUC expression in different types of ILD. Limited sample size, various types of diseases in ILD, and existing confounding factors are limitations of the current study. Large-scale studies are therefore still necessary.

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Competing interests None declared.

Patient consent for publication Not required.

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