Immunology Letters 128 (2010) 131–136

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/

Identification and clinical association of anti-cytokeratin 18 autoantibody in COPD

Yung-Bin Kuo[a,1], C. Allen Chang[a,1], Yao-Kuang Wu[b], Meng-Jer Hsieh[c], Chung-Hsien Tsai[d], Kuei-Tien Chen[e], Chun-Yu Chen[e], Err-Cheng Chan[e,*]

[a] College of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan
[b] Division of Pulmonary and Critical Care Medicine, Buddhist Tzu Chi General Hospital, Taipei, Taiwan
[c] Division of Pulmonary Infection and Immunology, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[d] Graduate Institute of Biochemical and Biomedical Engineering, Chang Gung University, Taoyuan, Taiwan
[e] Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan

ARTICLE INFO

Article history:
Received 8 September 2009
Received in revised form
27 November 2009
Accepted 11 December 2009
Available online 28 December 2009

Keywords:
COPD
Autoimmunity
Cytokeratin

ABSTRACT

The etiology of chronic obstructive pulmonary disease (COPD) remains unclear. A mechanism involving the autoimmune reaction in the pathogenesis of COPD has been proposed but not confirmed. The aim of this study was to investigate whether serum autoantibodies against pulmonary cellular proteins are present in COPD patients and to identify their autoantigens if possible. Samples from 50 COPD patients and 42 control subjects were studied. Circulating autoantibodies were detected by Western blot. Immunoprecipitation and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry were used to identify the autoantigens. Autoantibodies against pulmonary cellular antigens were found in the sera of COPD patients. Specifically, an autoantibody against the 45-kDa human cytokeratin 18 protein was found in 76.0% of COPD patients and 23.8% of control subjects ($p < 0.001$). Furthermore, the cytokeratin 18 autoantibody level was positively correlated with the FEV_1 (L) ($p = 0.013$) and FEV_1 (%pred.) ($p = 0.043$) values observed in COPD patients. This study identified the pulmonary epithelial cytokeratin 18 protein as a COPD-associated autoantigen and found that anti-cytokeratin 18 autoantibodies were prevalent in COPD patients. Our results support the hypothesis that humoral autoimmunity may be involved in the pathogenesis of COPD.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is predicted to become the fifth most common cause of disability worldwide by 2020 [1]. COPD is characterized by progressive airflow limitation that generally occurs due to a combination of chronic bronchitis and pulmonary emphysema [2]. According to the guidelines of the Global Initiative for Chronic Obstructive Lung Disease (GOLD), risk factors of COPD can be classified as either host factors (e.g., α1-antitrypsin deficiency) or environmental factors (e.g., tobacco smoking, occupational exposure to dust, air pollution, and infections) [3]. Among these, smoking is considered to be the most important risk factor associated with COPD development. Although smoking has been proven to induce airway inflammation and lung tissue destruction, these adverse effects persist even after smoking cessation for reasons that remain unclear [4–7]. This phenomenon suggests that pathological mechanisms other than smoking may be involved in the development of COPD [8].

The etiology of COPD is not completely understood. Some pathogenic mechanisms for COPD have been proposed, including oxidant–antioxidant imbalance, protease–antiprotease imbalance, immunological disorder, and cell repair mechanism deficiency. However, none of them has been able to satisfactorily explain the pathological changes of COPD [9]. An increasing number of studies have shown that adaptive immune response plays some role in the progression of this disease [10–14]. Moreover, several studies propose that an autoimmune mechanism may be involved in the pathogenesis of COPD [9,15–17]. A study by Lee et al. proved that

Abbreviations: COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease; FVC, forced vital capacity; FEV_1 (%), forced expiratory volume in one second; FEV_1 (%pred.), predicted forced expiratory volume in one second (%); FEV_1/FVC, absolute ratio of FEV_1 to forced vital capacity; BMI, body mass index; CRP, C-reactive protein; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; IP, immunoprecipitation; CK18, cytokeratin 18.

* Corresponding author at: Department of Medical Biotechnology and Laboratory Science, Chang Gung University, 259 Wen-Hua 1st Road, Kweishan, 333, Taoyuan, Taiwan. Tel.: +886 3 2118800x5220; fax: +886 3 2118741.
E-mail address: chanec@mail.cgu.edu.tw (E.-C. Chan).
[1] These two authors contributed equally to this study.

0165-2478/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2009.12.017

autoantibodies against extracellular components, such as elastin, are present in the sera of COPD patients [18]. Furthermore, Feghali-Btewart et al. recently reported that cytotoxic IgG autoantibodies against pulmonary epithelium are frequently observed in COPD patients [19].

The crucial pathologic features of COPD are chronic airway inflammation and the destruction of alveolar structure [8]. Autoimmunity may arise as a response to tissue injury, and unrestrained autoimmune reaction can evoke further inflammation and cause tissue damage [20]. Therefore, we propose that autoantibodies might be elicited during the development of COPD and that autoantigens might be present in airway epithelial cells. The aim of this study is to determine whether autoantibodies against airway epithelial cellular proteins exist in the sera of COPD patients and, if so, to identify the corresponding cellular autoantigens.

2. Methods and methods

2.1. Subjects

A total of 92 male subjects (50 COPD patients and 42 control subjects with normal spirometry) from Chang-Gung Memorial Hospital (Taoyuan, Taiwan) and Buddhist Tzu Chi General Hospital (Taipei, Taiwan) were enrolled. Two inclusion criteria for COPD were used: first, the ratio of post-bronchodilator (e.g., 400 μg salbutamol) forced expiratory volume in one second to the forced vital capacity (FEV_1/FVC) must be smaller than 0.7, and second, forced expiratory volume in one second (FEV_1) must be less than 80% of predicted value, as defined by GOLD [4]. Subjects with other pulmonary diseases (such as asthma, pneumonia, and lung cancer) were carefully excluded from this study. As study controls, 42 male individuals, including 22 healthy smokers (HS) and 20 non-smokers (NS), were recruited. All control subjects underwent thorough annual health examinations, had no history of airway disease, and presented normal lung function test results. All subjects received the same clinical assessments, including physical examination, lung function tests, and chest X-ray. None of the patients had been treated with corticosteroids during the 2 months prior to the study. Subjects with a smoking history equal to or greater than 10 pack-years were categorized as "smokers," while subjects who had stopped smoking equal to or greater than 2 years before the tests were defined as "ex-smokers (ES)." Non-smokers were defined as subjects who had never smoked. All study subjects signed forms indicating written consent. The study protocol was approved by the medical ethics and human clinical trial committees of Chang Gung Memorial Hospital and Buddhist Tzu Chi General Hospital.

2.2. Cell culture

Primary human pulmonary alveolar epithelial cells were purchased from ScienCell Research Laboratories (ScienCell, CA, USA). Cells were cultured in minimum essential medium supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). The cells were maintained in an incubator at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Pulmonary function test and chest radiography

All subjects underwent pulmonary function tests using the Microlab 3500 spirometer (Micro Medical, UK) according to published American Thoracic Society recommendations [21]. Chest radiographs (posteroanterior and lateral views) were performed at a standard 2-m focus-to-film distance according to previously reported radiographic criteria [22].

2.4. C-reactive protein assay

Concentrations of C-reactive protein (CRP) were assayed with a highly sensitive quantitative enzyme-linked immunosorbent assay kit (Alpha Diagnostics, TX, USA) following the manufacturer's instructions.

2.5. Western blot analysis

For autoantibodies screening, the recombinant human CK18 proteins (Progen Biotechnik, Germany) were used as antigens. Proteins were resolved by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA). After incubation with the individual sera, the immunoreactive bands were assessed by two independent investigators. To control for variation in the intensity of immunoreactive bands between batches, a reference serum was loaded and analyzed in each batch. A serum sample was considered positive if its immunoreactivity was equal to or greater than that of the positive control serum [23]. The relative intensities of immunoreactive bands were further analyzed by MultiGauge version 3.0 image analysis software (Fujifim, Japan) and quantitated in comparison with the reference serum.

2.6. Immunoprecipitation and mass spectrometry analysis

The immunoreactive cellular autoantigens were isolated as previously described [24]. The immunoprecipitated complexes were separated by 12% SDS-PAGE and visualized by silver staining. The target bands were then excised and subjected to in-gel trypsin digestion. The digested peptides were assayed with an ultraflex MALDI-TOF mass spectrometer (Bruker Daltonics, Germany), and the obtained mass spectral data were analyzed using the National Center for Biotechnology non-redundant protein database [24].

2.7. Statistical analysis

Statistical analyses were conducted using SPSS (version 12.0; IL, USA). A Chi-square test was applied to analyze characteristic differences. Differences between all groups were evaluated by the Kruskal–Wallis test, and differences between individual variables from two groups were evaluated by the non-parametric Mann–Whitney *U* test. Correlation between autoantibody levels and clinicopathological variables was calculated using Pearson correlation analysis. Statistical significance was defined as $p < 0.05$.

More detailed methods are provided in Supplementary data.

3. Results

3.1. Study subjects

The characteristics of all subjects are listed in Table 1. The mean age of the COPD patient group (75.4 ± 9.0; $p = 0.195$) was similar to that of the healthy smoker group (72.8 ± 9.3). The mean age of the healthy smoker group was not different from that of the non-smokers (70.8 ± 8.3; $p = 0.588$). The healthy smokers had approximate smoking history (pack-years) as COPD patients did.

3.2. Immunoscreening for anti-alveolar cellular protein autoantibodies

The alveolar cell lysates were used as the target antigens. Random serum specimens from the study subjects (control group: $n = 13$; COPD group: $n = 22$) were examined by preliminary immunoscreening. Fig. 1 shows that autoantibodies against cellular antigens were more frequently present in the serum of COPD

Table 1
Characteristics of study subjects.

	COPD groups ($n=50$)[a]			Control groups ($n=28$)	
	All patients (AP)	Ex-smokers (ES)	Current smokers (CS)	Healthy smokers (HS)	Non-smokers (NS)
Number	50	22	18	22	20
Age (years)	75.4 ± 9.0	76.6 ± 7.3	74.5 ± 11.0	72.8 ± 9.3	70.8 ± 8.3
GOLD stage					
I	4 (8%)	1 (4.6%)	3 (16.7%)	0	0
II	19 (38%)	7 (31.8%)	8 (44.4%)	0	0
III	23 (46%)	11 (50%)	7 (38.9%)	0	0
IV	4 (8%)	3 (13.6%)	0 (0%)	0	0
FEV_1 (%) predicted[†]	50.7 ± 21.9[*]	45.3 ± 22.1[*]	59.8 ± 23.3[*]	93.1 ± 17.5	93.4 ± 20.7
FEV_1/FVC (%)[†]	53.2 ± 12.1[*]	51.8 ± 14.0[*]	54.7 ± 12.2[*]	83.7 ± 6.0	84.7 ± 7.1
Smoking (pack-years)	47.9 ± 28.6	46.9 ± 24.9	51.1 ± 24.8	46.3 ± 24.9	0
BMI (kg/m^2)[†]	22.3 ± 4.2[*]	22.6 ± 4.1	21.8 ± 2.8[*]	24.3 ± 2.8	23.2 ± 2.6

Data shown are mean ± SD. GOLD: Global Initiative for Chronic Obstructive Lung Disease; FEV_1: forced expiratory volume in one second; FEV_1/FVC: absolute ratio of FEV_1 to forced vital capacity; BMI: body mass index.
[a] Among the COPD patients, 10 were smokers with smoking cessation of less than 2 years.
[*] $p < 0.05$ compared with healthy smokers (Mann–Whitney U test).
[†] $p < 0.05$, between all groups (Kruskal–Wallis test).

patients than in serum from the control subjects. In the COPD group, the molecular weight (kDa) and detection rate of the predominant immunoreactive cellular autoantigens were 98 (14%), 86 (18%), 75 (18%), 66 (14%), 51 (40%), 45 (45%), and 31 (14%), respectively. The detection rates of these immunoreactive bands in the control group were 8%, 0%, 15%, 8%, 23%, 8%, and 0%, respectively.

3.3. Identification of a 45-kDa cellular autoantigen

MALDI-TOF mass spectrometry was used to further characterize the most prevalent alveolar cellular autoantigen. The target cellular autoantigens were extracted by immunoprecipitation (IP), separated by 12% SDS-PAGE, and visualized by silver staining (Fig. 2). The 45-kDa immunoprecipitated autoantigen was further digested (in-gel) and analyzed by MALDI-TOF mass spectrometry. According to the results of a MASCOT search, the mass data of the 23 peptide fragments resulted from in-gel digestion matched human CK18 (accession number Swiss-Prot: PO5783, theoretical mass 48,029 Da), and the identified peptide sequences covered 58% of the total amino acid sequence of human CK18. The probability-based Mowse score, expected value, and calculated p*I* value were 224, 6.3e−19, and 5.34, respectively (Supplementary Table 1). Further confirmation of the 45-kDa autoantigen as human CK18 protein was achieved by immunoblot analysis using a specific monoclonal mouse anti-human CK18 antibody (CY-90, Sigma, MO, USA) (Fig. 3A and B). The immunoblot results showed that both the target 45-kDa cellular autoantigen and recombinant human CK18 protein were recognized by the monoclonal anti-CK18 protein antibody. The molecular weight of the immunoreactive band was also con-

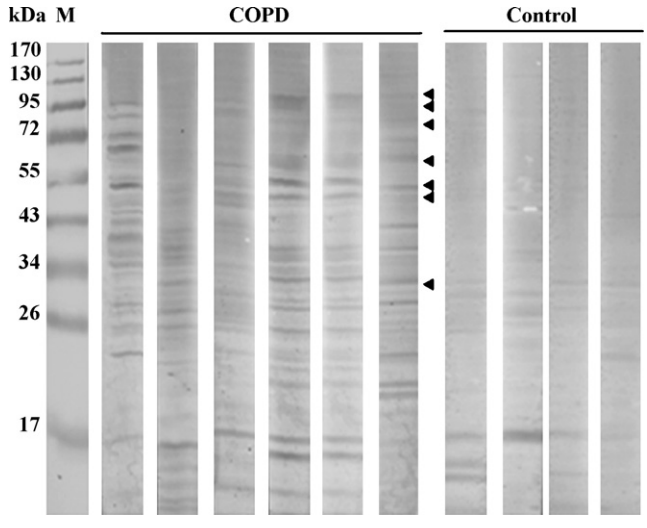

Fig. 1. A Western blot assay was used to detect anti-alveolar cellular protein autoantibodies in the sera of study subjects. Immunoreactive antibodies against alveolar cellular antigens were found more frequently in the sera of COPD patients than in the sera of control subjects. The arrows denote the locations of the more frequently found immunoreactive bands (98, 86, 75, 66, 51, 45, and 31 kDa). Each lane contains a serum specimen from an individual subject. COPD lanes: serum specimens from COPD subjects; control lanes: serum specimens from normal spirometry control subjects; lane M: standard molecular weight marker.

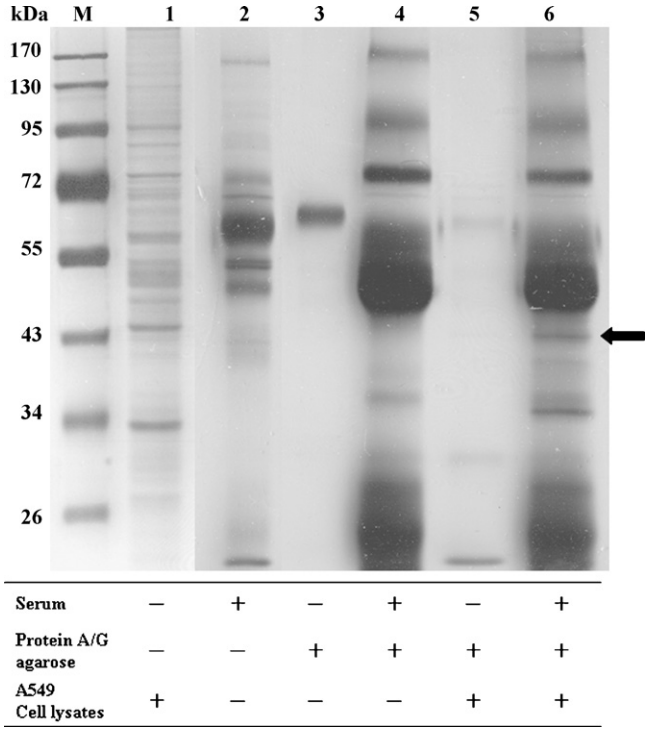

Fig. 2. Silver-stained SDS-PAGE gel of immunoprecipitation reactions. The 45-kDa target protein isolated and identified using MALDI-TOF mass spectrometry is indicated with an arrow. Lane M: molecular weight standard markers; lanes 1–5: experimental controls; lane 6: one COPD patient serum specimen immunoprecipitated with alveolar cellular antigens; + present; − absent.

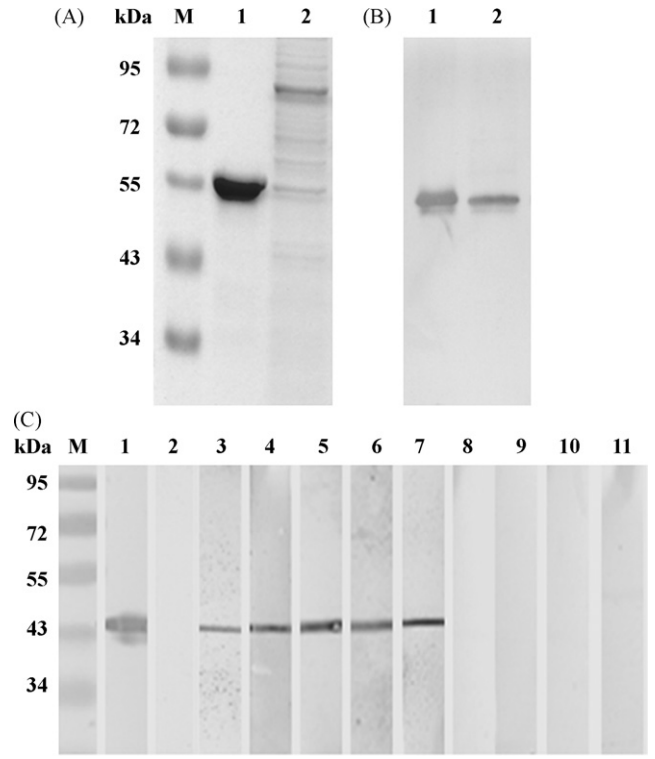

Fig. 3. Immunoscreening of anti-cytokeratin 18 (CK18) autoantibodies, identification of the 45-kDa target autoantigen as human CK18 protein, and confirmation of the presence of anti-CK18 autoantibodies in the sera of COPD patients. (A) SDS-PAGE of primary human alveolar epithelial cells lysates (lane 1) and recombinant human CK18 protein (lane 2). (B) A Western blot shows that the target 45-kDa cellular autoantigen of primary human alveolar epithelial cells (lane 1) and recombinant human CK18 protein (lane 2) could be recognized by a mouse monoclonal anti-CK18 antibody. (C) Western blot detection of autoantibodies against recombinant human CK18 protein in the sera of study subjects. Each lane contains a serum specimen from an individual subject. Lane 1: positive control; lane 2: negative control; lanes 3–7: serum specimens from COPD subjects; lanes 8–11: serum specimens from control subjects; lane M: molecular weight standard marker.

sistent with previous results. Taken together, we confirmed that the target 45-kDa autoantigen that reacts with the autoantibodies in the sera of COPD patients was human CK18 protein.

3.4. The prevalence of anti-cytokeratin 18 autoantibodies

The presence of anti-CK18 autoantibodies in the sera of COPD patients was determined by using recombinant human CK18 protein (Progen, Germany) as an antigen. Fig. 3C shows the prevalence of anti-CK18 autoantibodies among the study subjects. Autoantibodies to CK18 protein were detected more frequently in COPD patients (38/50; 76%) than in control subjects (10/42; 23.8%; $p < 0.001$).

3.5. Quantification of anti-cytokeratin 18 autoantibodies

We found that the levels of anti-CK18 autoantibody were significantly higher in the COPD group (38.01 ± 30.73) than in controls group (14.84 ± 13.88; $p < 0.001$) (Fig. 4). Among COPD patients, there was no significant difference between the levels of anti-CK18 autoantibody in ex-smokers and current smokers (47.28 ± 39.09 *versus* 33.54 ± 23.33; $p = 0.198$). In addition, the levels of anti-CK18 autoantibody were not significantly different between healthy smokers and non-smokers (17.19 ± 16.04 *versus* 12.26 ± 10.85; $p = 0.273$).

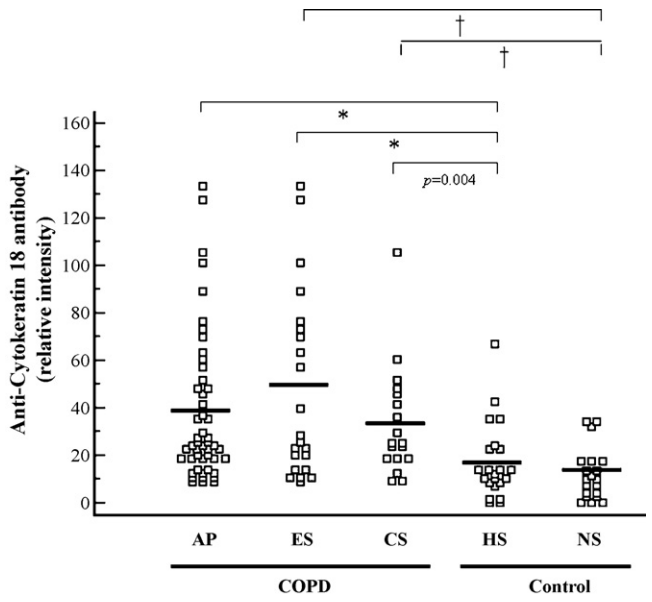

Fig. 4. Analysis of anti-cytokeratin 18 autoantibody levels in subdivided study groups. Serum specimens from all COPD patients (AP), ex-smokers with COPD (ES), current smokers with COPD (CS), healthy smokers (HS), and healthy non-smokers (NS) were tested. The levels of anti-CK18 autoantibodies are presented as intensity relative to a control marker. Differences between pairs of groups were compared using the Mann–Whitney *U* test. Horizontal bars indicate mean values. $^{*}p < 0.001$ compared with healthy smokers group; $^{\dagger}p < 0.001$ compared with non-smokers group.

3.6. The correlation between anti-cytokeratin 18 autoantibody levels and clinical parameters

In the COPD group, the levels of anti-CK18 autoantibody (i.e., the relative intensity of the Western blot band) were significantly related to clinical parameters, such as FEV_1 (L) ($r_s = -0.349$, $p = 0.013$; Fig. 5A) and FEV_1 (%) predicted values ($r_s = -0.288$, $p = 0.043$; Fig. 5B), but were not associated with other clinical parameters, such as age ($r_s = -0.073$; $p = 0.614$), smoking pack-years ($r_s = -0.011$; $p = 0.941$), FEV_1 (%) predicted/FVC ($r_s = -0.211$; $p = 0.142$), CRP levels ($r_s = 0.004$; $p = 0.984$), or body mass index (BMI) ($r_s = -0.174$; $p = 0.271$).

4. Discussion

The possibility that an autoimmune response is involved in the pathogenesis of COPD has been proposed in several previous studies [9,17,25,26]. According to recent reports, the extracellular matrix protein elastin and an unidentified 130-kDa protein were discovered as autoantigens associated with COPD [18,19]. The present study aimed to test whether any pulmonary cellular proteins are potential autoantigens associated with COPD. Intriguingly, we have successfully identified the human CK18 protein as a 45-kDa cellular autoantigen. Our results showed that the levels of anti-CK18 autoantibody in COPD patients were significantly higher than those in healthy controls were.

Cigarette smoking not only induces apoptosis in airway epithelial cells but also exerts several other adverse effects on the host immune system [27–29]. In order to assess the effect of smoking status on the levels of anti-CK18 antibody, we examined serum samples from groups with different smoking status. No significant difference was found between non-smokers and healthy smokers ($p = 0.273$) or between ex-smokers and current smokers with COPD ($p = 0.532$). Moreover, no significant correlation has been found between autoantibody levels and smoking history among the COPD patients group ($p = 0.941$) or among all smokers (i.e., the current

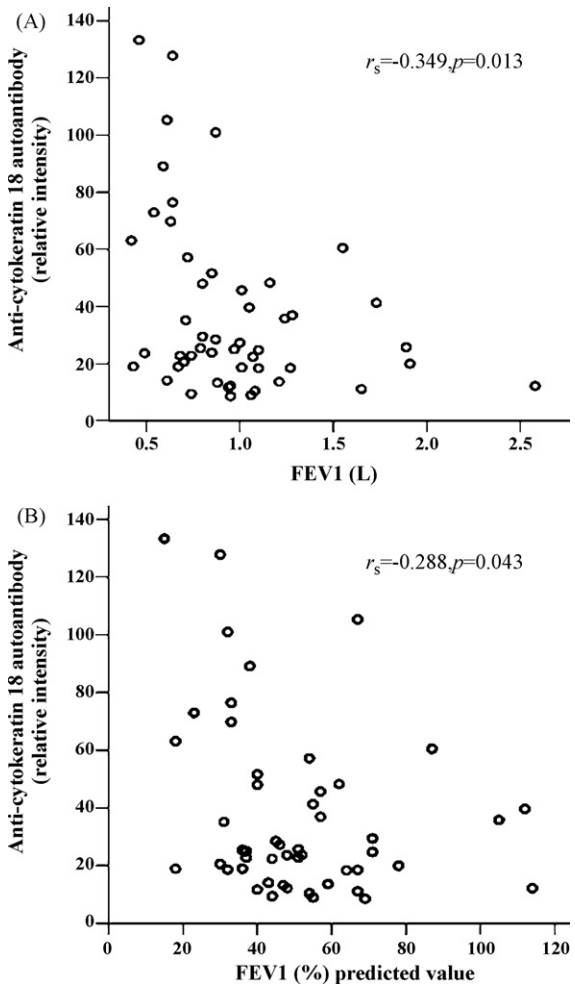

Fig. 5. Correlation of anti-cytokeratin 18 autoantibody levels with FEV_1 (L) and FEV_1 (%) predicted in COPD patients. The levels of anti-CK18 autoantibodies are presented as intensity relative to a control marker. Correlations between FEV_1 (L) (Fig. 5A) and FEV_1 (%) predicted (Fig. 5B) with the levels of anti-CK18 autoantibodies in serum from COPD patients were evaluated with Pearson correlation analysis.

smoker (CS), ES, and HS groups, $p = 0.771$) (Spearman's rank correlation coefficient). These results are consistent with the findings of previous investigations that showed that smoking status did not affect the prevalence of anti-epithelial antibodies [19]. Thus, cigarette smoking may not be related to anti-CK18 autoantibody levels.

The immunological mechanisms that drive the formation of anti-CK18 autoantibody in COPD remain unknown. It is worth noting that defects in apoptosis could induce inflammation and autoimmune reactions [28,30,31]. Many studies have shown that abnormal immune responses and apoptosis-associated defects are present in COPD patients [4,10,28]. Further investigations were needed to clarify that whether these defects play important roles in autoantibodies production in COPD.

The source of cytokeratin 18 in the serum of COPD as well as the mechanisms of release from pulmonary epithelium have long been unclear. Recent evidence suggests that circulation cytokeratins are released from dying tumor cells [43,44], and CK18 have also been used as a biomarker for monitoring of disease progression in lung cancer patients [45,46]. To eliminate the interference from apoptotic or necrotic tumour cells, in this study, we carefully excluded the patients with malignant and other relevant diseases. Cytokeratins are a group of intermediate filament proteins (keratins) found in the intra-cytoplasmic cytoskeleton of epithelial tissue. Cytokeratin 18, the type I cytokeratin protein, is primarily expressed in epithelial tissues [32,33]. Interestingly, CK18 protein was also found in pulmonary bronchial and alveolar epithelial cells, which are the major disease sites of COPD [34]. Although CK18 protein is normally located in the cytoplasm, CK18 neoepitopes may be released into circulation following epithelial cell apoptosis (cleaved by caspase 3); the resulting antibody–antigen interaction could significantly promote COPD pathology [35,36]. Further studies will be needed to clarify this line of reasoning. Moreover, CK18 protein has previously been identified as the target autoantigen associated with several diseases, including non-allergic bronchial asthma, idiopathic pulmonary fibrosis, autoimmune hepatitis, and rheumatoid arthritis. This may imply that the anti-CK18 autoantibody is not specific to COPD and may be involved in a general response to tissue injury [37–39]. Given our findings and the results of previous studies [18,19,40], it is plausible that autoantibodies, at least in part, participate in the development of COPD, as recently hypothesized [17,26]. The exact roles of autoantibodies in the pathogenesis of COPD need to be investigated further.

A specific autoantibody marker is helpful in determining the prognosis and severity of the related disease [41]. In our study, we also investigated the correlation between airway obstruction severity and anti-CK18 autoantibody levels in COPD. Our results showed a significant negative correlation between anti-CK18 autoantibody levels and FEV_1 (L) and FEV_1 (%) predicted value. Thus, measuring the concentration of anti-CK18 antibody could assist in the clinical evaluation of treatment response or prognosis of COPD.

While COPD is a complex chronic inflammatory disease of the lungs, it also manifests itself in many systemic extrapulmonary symptoms [6,42]. However, the relationships between elevated anti-CK18 autoantibody levels and systemic chronic inflammation or other extrapulmonary effects in COPD patients remain largely unknown. Currently, CRP concentration and BMI are used to evaluate inflammation and systemic manifestation of COPD, respectively. Therefore, the CRP concentrations and BMI values of these COPD patients were determined to investigate whether these two factors are associated with the levels of anti-CK18 autoantibodies. Unfortunately, no significant correlation was found. This is in contrast to previous results in which the presence of autoantibodies against the unidentified 130-kDa cellular antigen was coupled with low BMI value in COPD patients [19], suggesting that these autoantigens may work through different systemic pathways.

One of the limitations of our study was our inability to precisely determine whether the existence of the anti-CK18 autoantibody is the "cause" or the "consequence" of lung tissue damage in COPD patients. Further studies including the development of an animal model for autoimmune COPD are needed to determine the role of autoimmune responses in the pathogenesis of COPD.

In Conclusion, we reported that cytokeratin 18 is a pulmonary cellular autoantigen associated with COPD and that circulating anti-cytokeratin 18 autoantibodies are present in the sera of COPD patients. Our study provides greater evidence to support the hypothesis that autoimmune responses may participate in the pathogenesis of COPD. These findings may prove helpful in the development of new diagnostic and therapeutic methods for better management of COPD.

Acknowledgements

The authors thank Dr. Ying Huang Tsai for his expert advice and the Proteomics Core Lab at Chang Gung University, Taoyuan, Taiwan for technical assistance with the MALDI-TOF mass spectrometry analyses. The study was supported by Chang Gung

Medical Research Grants CMRP150201 and CMRP160261 and the National Science Council of the Republic of China (Taiwan) Grant number NSC962113M009019-MY2 (to CAC).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.imlet.2009.12.017.

References

[1] Lopez AD, Shibuya K, Rao C, Mathers CD, Hansell AL, Held LS, et al. Chronic obstructive pulmonary disease: current burden and future projections. Eur Respir J 2006;27:397–412.
[2] Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med 2001;163:1256–76.
[3] Ramsey SD, Hobbs FD. Chronic obstructive pulmonary disease, risk factors, and outcome trials: comparisons with cardiovascular disease. Proc Am Thorac Soc 2006;3:635–40.
[4] Hodge S, Hodge G, Holmes M, Reynolds PN. Increased airway epithelial and T-cell apoptosis in COPD remains despite smoking cessation. Eur Respir J 2005;25:447–54.
[5] Celli BR, MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J 2004;23:932–46.
[6] Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax 2004;59:574–80.
[7] Agusti A, Thomas A. Neff lecture. Chronic obstructive pulmonary disease: a systemic disease. Proc Am Thorac Soc 2006;3:478–81.
[8] Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 2004;350:2645–53.
[9] Agusti A, MacNee W, Donaldson K, Cosio M. Hypothesis: does COPD have an autoimmune component? Thorax 2003;58:832–4.
[10] Cosio MG, Guerassimov A. Chronic obstructive pulmonary disease. Inflammation of small airways and lung parenchyma. Am J Respir Crit Care Med 1999;160:S21–5.
[11] Richmond I, Pritchard GE, Ashcroft T, Avery A, Corris PA, Walters EH. Bronchus associated lymphoid tissue (BALT) in human lung: its distribution in smokers and non-smokers. Thorax 1993;48:1130–4.
[12] Bosken CH, Hards J, Gatter K, Hogg JC. Characterization of the inflammatory reaction in the peripheral airways of cigarette smokers using immunocytochemistry. Am Rev Respir Dis 1992;145:911–7.
[13] Casolaro MA, Bernaudin JF, Saltini C, Ferrans VJ, Crystal RG. Accumulation of Langerhans' cells on the epithelial surface of the lower respiratory tract in normal subjects in association with cigarette smoking. Am Rev Respir Dis 1988;137:406–11.
[14] Plumb J, Smyth LJ, Adams HR, Vestbo J, Bentley A, Singh SD. Increased T-regulatory cells within lymphocyte follicles in moderate COPD. Eur Respir J 2009;34:89–94.
[15] Sullivan AK, Simonian PL, Falta MT, Mitchell JD, Cosgrove GP, Brown KK, et al. Oligoclonal CD4+ T cells in the lungs of patients with severe emphysema. Am J Respir Crit Care Med 2005;172:590–6.
[16] Taraseviciene-Stewart L, Scerbavicius R, Choe KH, Moore M, Sullivan A, Nicolls MR, et al. An animal model of autoimmune emphysema. Am J Respir Crit Care Med 2005;171:734–42.
[17] Cosio MG, Saetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. N Engl J Med 2009;360:2445–54.
[18] Lee SH, Goswami S, Grudo A, Song LZ, Bandi V, Goodnight-White S, et al. Antielastin autoimmunity in tobacco smoking-induced emphysema. Nat Med 2007;13:567–9.
[19] Feghali-Bostwick CA, Gadgil AS, Otterbein LE, Pilewski JM, Stoner MW, Csizmadia E, et al. Autoantibodies in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008;177:156–63.
[20] Davidson A, Diamond B. Autoimmune diseases. N Engl J Med 2001;345:340–50.
[21] Standardization of Spirometry, 1994 update. American Thoracic Society. Am J Respir Crit Care Med 1995;152:1107–36.
[22] Muller NL, Coxson H. Chronic obstructive pulmonary disease. 4. Imaging the lungs in patients with chronic obstructive pulmonary disease. Thorax 2002;57:982–5.
[23] Berger T, Rubner P, Schautzer F, Egg R, Ulmer H, Mayringer I, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. N Engl J Med 2003;349:139–45.
[24] Wu CC, Chen HC, Chen SJ, Liu HP, Hsieh YY, Yu CJ, et al. Identification of collapsin response mediator protein-2 as a potential marker of colorectal carcinoma by comparative analysis of cancer cell secretomes. Proteomics 2008;8:316–32.
[25] Taraseviciene-Stewart L, Douglas IS, Nana-Sinkam PS, Lee JD, Tuder RM, Nicolls MR, et al. Is alveolar destruction and emphysema in chronic obstructive pulmonary disease an immune disease? Proc Am Thorac Soc 2006;3:687–90.
[26] Tzortzaki EG, Siafakas NM. A hypothesis for the initiation of COPD. Eur Respir J 2009;34:310–5.
[27] Sopori M. Effects of cigarette smoke on the immune system. Nat Rev Immunol 2002;2:372–7.
[28] Hodge S, Hodge G, Scicchitano R, Reynolds PN, Holmes M. Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. Immunol Cell Biol 2003;81:289–96.
[29] Rutgers SR, Postma DS, ten Hacken NH, Kauffman HF, van Der Mark TW, Koeter GH, et al. Ongoing airway inflammation in patients with COPD who do not currently smoke. Chest 2000;117:262S.
[30] Patel VA, Longacre A, Hsiao K, Fan H, Meng F, Mitchell JE, et al. Apoptotic cells, at all stages of the death process, trigger characteristic signaling events that are divergent from and dominant over those triggered by necrotic cells: implications for the delayed clearance model of autoimmunity. J Biol Chem 2006;281:4663–70.
[31] Krysko DV, D'Herde K, Vandenabeele P. Clearance of apoptotic and necrotic cells and its immunological consequences. Apoptosis 2006;11:1709–26.
[32] Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 1982;31:11–24.
[33] Steinert PM. Structure, function, and dynamics of keratin intermediate filaments. J Invest Dermatol 1993;100:729–34.
[34] Blobel GA, Moll R, Franke WW, Vogt-Moykopf I. Cytokeratins in normal lung and lung carcinomas. I. Adenocarcinomas, squamous cell carcinomas and cultured cell lines. Virchows Arch B Cell Pathol Incl Mol Pathol 1984;45:407–29.
[35] Leers MP, Kolgen W, Bjorklund V, Bergman T, Tribbick G, Persson B, et al. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. J Pathol 1999;187:567–72.
[36] Caulin C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. J Cell Biol 1997;138:1379–94.
[37] Nahm DH, Lee YE, Yim EJ, Park HS, Yim H, Kang Y, et al. Identification of cytokeratin 18 as a bronchial epithelial autoantigen associated with nonallergic asthma. Am J Respir Crit Care Med 2002;165:1536–9.
[38] Murota M, Nishioka M, Fujita J, Dobashi N, Wu F, Ohtsuki Y, et al. Anti-cytokeratin antibodies in sera of the patients with autoimmune hepatitis. Clin Exp Immunol 2001;125:291–9.
[39] Dobashi N, Fujita J, Murota M, Ohtsuki Y, Yamadori I, Yoshinouchi T, et al. Elevation of anti-cytokeratin 18 antibody and circulating cytokeratin 18: anti-cytokeratin 18 antibody immune complexes in sera of patients with idiopathic pulmonary fibrosis. Lung 2000;178:171–9.
[40] Leidinger P, Keller A, Heisel S, Ludwig N, Rheinheimer S, Klein V, et al. Novel autoantigens immunogenic in COPD patients. Respir Res 2009;10:20.
[41] ter Borg EJ, Horst G, Hummel EJ, Limburg PC, Kallenberg CG. Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long-term, prospective study. Arthritis Rheum 1990;33:634–43.
[42] Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J 2009;33:1165–85.
[43] Linder S. Cytokeratin markers come of age. Tumour Biol 2007;28:189–95.
[44] Barak V, Goike H, Panaretakis KW, Einarsson R. Clinical utility of cytokeratins as tumor markers. Clin Biochem 2004;37:529–40.
[45] Nisman B, Lafair J, Heching N, Lyass O, Baras M, Peretz T, et al. Evaluation of tissue polypeptide specific antigen, CYFRA 21-1, and carcinoembryonic antigen in nonsmall cell lung carcinoma: does the combined use of cytokeratin markers give any additional information? Cancer 1998;82:1850–9.
[46] Linder S, Havelka AM, Ueno T, Shoshan MC. Determining tumor apoptosis and necrosis in patient serum using cytokeratin 18 as a biomarker. Cancer Lett 2004;214:1–9.