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

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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.

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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