Case Report

A case of non-specific interstitial pneumonia associated with primary lung cancer: possible role of antibodies to lung cancer cells in the pathogenesis of non-specific interstitial pneumonia

I. Yamadori*, T. Sato*, J. Fujita†, N. Dobashi†, Y. Ohtsuki† and J. Takahara†

* National Okayama Hospital, Okayama, Japan
† First Department of Internal Medicine, Kagawa Medical University, Kagawa, Japan
‡ Second Department of Pathology, Kochi Medical School, Kochi, Japan

Introduction

Bronchogenic carcinoma has frequently been associated with para-neoplastic phenomena, ranging from mild systemic or cutaneous disease to hypercoagulability and severe neuromyopathic disorders (1). Non-specific interstitial pneumonia (NIP) was first described by Katzenstein and Fiorelli in 1994 (2). It has also been reported that NIP has been associated with collagen vascular disorders (2). We report a case of primary lung cancer associated with NIP and hypothesize that anti-human lung cancer cell antibodies play a role in the pathogenesis of NIP.

Case report

A 68-year-old man with a 2-month history of progressive dyspnoea on exertion attended the outpatient clinic. He had a dry cough and dyspnoea, but no sputum or fever. He was an office worker, who had not experienced any risk factors for occupational or environmental exposure to toxic materials, nor did he have hypersensitivity pneumonitis. His previous medical history was unremarkable except for smoking of 40 pack-years. There was no abnormal finding in chest X-ray which was taken one year ago. A rapidly symptomatic progression of his dyspnoea occurred 2 weeks after attending the outpatient clinic and he was subsequently admitted to hospital. On physical examination, he was afebrile with a respiratory rate of 25 min⁻¹, blood pressure of 110/70 mmHg⁻¹, and a regular pulse rate of 84 min⁻¹. The chest was symmetric and bibasilar and coarse crackles were auscultated. Laboratory data showed a normal blood count without eosinophilia. The electrolyte, renal and liver function tests were all normal. Arterial blood gas analysis showed \( \text{PO}_2 \) of 78 mmHg, \( \text{A-aDO}_2 \) of 32 mmHg and pH 7.45. Lung function studies showed a vital capacity of 61.4% and forced expiratory volume in 1 s (FEV₁) 81.3%.

Chest X-ray and computerized tomography (CT) revealed a diffuse bilateral interstitial infiltrate as well as a nodule (sized 2.0 x 2.0 cm) in the right upper lobe (Fig. 1). Flexible bronchoscopy was non-diagnostic. The right side of the lung was opened at S3 (distant from the lung tumour) and needle biopsy of the tumour (right S2) performed. Pathological evaluation revealed squamous cell lung cancer in the right S2 as well as NIP (group II) in the right S3. Immunohistochemical staining of squamous cell lung cancer of the right upper lobe using monoclonal antibodies against several cytokeratins demonstrated cytokeratin 8, 17, 18 and 19.

Although the lung cancer was clinically staged at 1, the patient refused surgical treatment. Oral prednisolone (1 mg kg⁻¹ day⁻¹) was initiated, and the bilateral infiltrating shadow disappeared. No exacerbation of interstitial pneumonia was observed throughout more than 1 yr oral prednisolone treatment.

To evaluate the existence of antibodies against lung proteins, Western immunoblot analysis was performed. SDS-polyacrylamide gel electrophoresis was performed according to Laemmli’s method (3) with a slight modification. Cell lysates of several lung cancer cell lines were then applied to a 10/20% SDS polyacrylamide gel, electrophoresed (60 mA, 120 min). Proteins were then electrophoretically transferred onto a nitrocellulose membrane using the method of Towbin et al. (4). Proteins were detected by immunoblotting, using a patient’s serum (1:50 dilution), peroxidase conjugated goat anti-human IgG antibody (Sigma Immuno Chemicals, lot 094H-4810, St Louis, MO, U.S.A.), and stained with 4 CN PLUS for chromogenic detection of horseradish peroxidase (NEM™ Life Science Products, Boston, MA, U.S.A.). Western immunoblot analysis showed that several antibodies against human lung cancer cell lines were found in this patient’s sera (Fig. 2). Among them, both anti-cytokeratin 8 antibody and anti-cytokeratin 19 antibody were characterized. However, one antibody against an unknown antigen was measured.
LUNG CANCER AND NIP

Fig. 1. Chest computerized tomography (CT) findings on admission. Bilateral interstitial consolidation and a coin lesion (arrow) are demonstrated.

Fig. 2. Western immunoblot analysis using the patient's sera against lysates of several lung cancer cell lines. Lane 1; recombinant human cytokeratin 19, lane 2; Recombinant human cytokeratin 8, lane 3; A549 cell line (adenocarcinoma), lane 4; PC9 (adenocarcinoma), lane 5; PC3 (adenocarcinoma), lane 6; RERF-LC-OK (adenocarcinoma), lane 7; LC1/SQ (squamous cell lung cancer), lane 8; recombinant human cytokeratin 8, lane 9; recombinant human cytokeratin 19. The bands correspond cytokeratin 8 with increased amounts of carbohydrates (our preliminary data, arrow *), cytokeratin 8 (arrow **), unidentified antigen (arrow ***), and cytokeratin 19 (arrow ****). Numbers on the left side represent molecular weight markers.

Discussion

In this report, we described a case of lung cancer which is complicated with NIP. To our knowledge this is the first description of an association between NIP and lung cancer. In this patient's serum, we also demonstrated antibodies to lung cancer cell lines, including anti-cytokeratin 8 as well as anti-cytokeratin 19.

In 1994, Katzenstein and Fiorelli reported the histological features and clinical significance of NIP (2). The histological features of NIP include a varying degree of interstitial inflammation and fibrosis which appear to develop over a specific time (i.e., the process is temporarily uniform) (2). In addition, they also reported that 10 of their 64 patients with NIP had an association with connective tissue diseases, including two patients with polymyositis (2).

They also reported that the lesion of cellular interstitial pneumonia associated with dermatomyositis termed by Tazelaar et al. (5), corresponds well to that of NIP. This evidence suggests that the pathogenesis of NIP closely relates to autoimmune disorders.

In the present study, we demonstrated anti-cytokeratin 8 and 19 antibodies in the patient's sera. Cytokeratin, which is one of the five different intermediate filaments, is characteristically expressed in epithelial cells (6). About 20 different cytokeratin polypeptides have been identified in human epithelial cells (6). It has been reported that antibodies to cytoskeletal proteins are associated with some organ-specific autoimmune diseases (7). Cytokeratin-specific antibodies appear to increase in diseases such as alcoholic liver disease and rheumatic disorders (8, 9), and several authors subscribe to the idea that anti-cytokeratin antibodies are formed in response to epithelial cell injury or death (10–13). However, none of these reports have described autoantibodies as specific to cytokeratin 8 or 19.

The significance of anti-cytokeratin 8 and 19 autoantibodies in this patient in the pathogenesis of NIP should also be discussed. The squamous cell lung cancer in this patient strongly expressed cytokeratin 8, 17, 18 and 19. There is a close temporal relationship between the diseases, together with neoplastigenic stimulation resulting in antibody formation or immune complex deposition in the lung and concomitantly in NIP. This is further supported by previous research which links lung cancer to other remote paraneoplastic syndromes (1).

Iyonaga et al. revealed that hyperplastic type II cells strongly express cytokeratins 7, 8, and 19 (14). In addition, some studies have shown that antibodies can penetrate cells in vivo (15). Therefore, antibodies which bind to cytokeratin 8 and 19 may be pathologically significant, since defects in cytoskeletal organization are known to be capable of disrupting cell function of hyperplastic type II cells (16). In addition, cytokeratin 8 and 19 may be released from cancer cells as well as epithelial cells following cell injury. The resulting antibody-antigen interaction with immune complex formation could have a significant role in the perpetuation of the disease process, either by direct injury of epithelial cells or via local macrophage activation as they are cleared by phagocytosis (17).

The possibility that other types of anti-cytokeratin antibodies exist in this patient cannot be excluded. However, we used several lung cancer cell lines and the patient's serum only reacted to 54 kD (cytokeratin 8), 45 kD (unknown, possibly cytokeratin 18) and 40 kD (cytokeratin 19) proteins. Therefore, we believe that there are no significant antibodies against other cytokeratins.

We could not rule out the possibility that the existence of cytokeratin 8 and 19 autoantibodies were a non-specific consequence of lung injury. Therefore, the significance of anti-cytokeratin 8 and 19 antibodies in the pathogenesis of NIP should be evaluated in future studies. In addition, although we could characterize two antigens as cytokeratin 8 and 19, the other antigen should be identified in future studies.
In summary, we present a case of lung cancer associated with NIP. Anti-human lung cancer cell antibodies including anti-cytokeratin 8 and 19 antibodies may have played a role in the pathogenesis of NIP.

References


