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Original Article

# Presence of immunoglobulin heavy chain rearrangement in so-called "plasma cell granuloma of the lung"

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SUMMARY

Inflammatory pseudotumor of the lung appears to be a set of heterogeneous disorders. Histologically, three subtypes of pulmonary IPTs have been delineated. Among these, plasma cell granuloma (PCG) is characterized by prominent lymphoplasmacytic infiltration, and PCG has been added to the list of differential diagnostic problems of mucosa-associated lymphoid tissue (MALT) type lymphoma. To investigate the presence or absence of monoclonal B-cell proliferation, we analyzed the immunohistological and genotypic findings in three cases of pulmonary PCGs. Histologically, the three lesions were characterized by severe infiltration of mature plasma cells, plasmacytoid cells, and small lymphocytes intermixed. Scattered Russell bodies (intracytoplasmic inclusions) were present in all three cases, but there were no Dutcher bodies (intranuclear inclusions) or centrocyte-like cells. Immunohistochemical studies of light chain determinants demonstrated the polytypic nature of B-cells. There was no $CD5^+$, $CD43^+$ or cyclin $D1^+$ B-lymphocytes in any of the three lesions. There were no lymphoepithelial lesions detected within any of the three lesions even by immunostaining for cytokeratin. However, polymerase chain assay for immunoglobulin heavy chain gene demonstrated a clonal band in one of the three cases. It currently remains unclear whether this one case, demonstrating IgH gene rearrangement in our series, could be a sign of the prelymphomatous stage (e.g. incipient MALT type lymphoma) or merely represents an exaggeration of normal B-cell clonal response.



## Introduction

So-called "Inflammatory pseudotumors (IPTs)" affect almost all major organs including the lungs [3,20]. Histologically, IPTs are characterized as an irregular proliferation of myofibroblasts intermixed with inflammatory cells, mainly lymphocytes and plasma cells [3,20]. Histologically, IPT of the lung has been classified into three histological types: (i) fibrohistiocytic type, (ii) plasma cell granuloma (PCGs), and (iii) largely sclerosed or fibrosed type [20]. True neoplastic proliferations of mesenchymal or dendritic cells have been reported in some IPTs [2,3,13,21]. Pulmonary IPTs have also been associated with previous viral infections such as human herpes virus type-8 (HHV-8) [8]. Recently, Zen et al. [22] demonstrated that some pulmonary PCGs represent an IgG4-related sclerosing disease. Rarely, pulmonary IPTs have also been associated with B-cell lymphoma [13]. Moreover, PCG has been added to the list of differential diagnostic problems of extramedullary plasmacytoma (EMP) [12]. To examine the presence or absence of monoclonal B-cell proliferation, we analyzed the immunohistological and genotypic findings in three cases of pulmonary PCGs.

## Materials and methods

The tissue specimens were fixed in formalin solution, routinely processed and embedded in paraffin. For light microscopic examination, the sections were stained with hematoxylin–eosin (HE) and elastica van Gieson (EVG) stain.

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Immunohistochemical studies were performed using the antigen retrieval method on the avidin–biotin–peroxidase method or Ventana automated (BenchMark™) stainer according to the manufacturer's instructions.

The panel of antibodies included human immunoglobulin light chains (kappa and lambda) (Dako A/S, Glostrup, Denmark), IgG (Novocastra, Newcastle, UK), IgA (Novocastra), IgM (Novocastra), MCO011 (IgG4;Binding Site, Birmingham, UK), PS-1 (CD3; Immunotech, Marseille, France), 4C7 (CD5; Novocastra), L26 (CD20; Dako), a cocktail of 2G9 (CD21; Novocastra) and RB L25 (CD35; Novocastra), 1B12 (CD 23; Novocastra), DFT-1 (CD43; Dako), 1B16 (CD56; Novocastra), PGM-1(CD68; Dako), 5A4 (CD246 [anaplastic lymphoma kinase, ALK]; Novocastra), SP4 (Cyclin D1; Nichirei Co., Tokyo, Japan), AE1/3 (Dako), V9 (vimentin; Dako), D33 (desmin; Dako), HHF35 (muscle-specific actin; Nichirei Co.), S-100 (Dako), and 137B1 (human herpes virus type-8, Novocastra). Sections with known reactivity for antibodies assayed served as positive controls, and sections treated with normal rabbit- and mouse serum served as negative controls.

In situ hybridization (ISH) with Epstein-Barr virus (EBV)-encoded small RNA (EBER) oligonucleotides was performed to test for the presence of EBV small RNA in formalin-fixed, paraffin-embedded sections using a Ventana automated (BenchMark™) stainer.

DNA was extracted from the paraffin-embedded section. The variable region (CDR2 and FW3) and VDJ region (CDR3) of the immunoglobulin heavy chain (IgH) gene were amplified by semi-nested PCR, using primers of FR2B, LJH, and VLJH, according to a previously described method [14]. Primers were as follows: 5′-CCGG(A/G)AA(A/G)(A/G) GTCTGGAGTGG-3′, as up-stream consensus V region primer (FR2B); 5′-TGAGGAGACGGTGACC-3′, as a consensus J region primer (LJH); 5′-GTGACCAGGGT [A/C/G/T] CCTTGGCCCCAG-3′, as a consensus J region primer (VLJH). PCR products were estimated to be about 200–300 bps in length.

The API2-MALT1 fusion transcript was examined using formalin-fixed and paraffin-embedded tissue according to the method recently described by us [11].

## Results

### *Clinical findings*

The clinical histories of PCG are summarized in Table 1. Only one (no.1) of three cases showed bloody sputum. The remaining two cases (nos. 2 and 3) were asymptomatic but had shown abnormal shadows on chest radiograph during a medical check-up. Chest radiographs and computed tomography demonstrated a solitary nodule in the peripheral pulmonary field in two cases (nos. 1 and 3) and four nodules on the peripheral pulmonary field of the bilateral lobes in the remaining one case (no. 2). One patient (no. 3) had a history of rectal cancer before the episode of PCG. Two cases (nos. 2 and 3) showed mediastinal lymphadenopathy. There was no other evidence of disease in any of the three cases.

Elevated serum IgG level had been recorded in one (no. 2) of the three cases. Antinuclear antibody was detected in Case 2. Serum IgG4 level was within the normal range in of two cases (nos. 1 and 3) examined.

Clinically, two cases (nos. 1 and 2) were suspected of having primary lung cancer and Case 3 was suspected of having metastatic rectal cancer. Two cases (nos. 2 and 3) showed hilar lymphadenopathy. However, lymph node biopsies were not performed.

### *Pathological findings*

Macroscopically, two lesions (nos. 1 and 3) were solitary tan and firm and were relatively well circumscribed without fibrosis (Fig. 1a). The remaining case comprised four nodules in the bilateral lungs.

Histologically, the lesions were characterized by a relatively well-demarcated mass composed of a few lymphoid follicles and chronic inflammatory process intermixed with irregular fibrosis (Fig. 1b). The lesions demonstrated severe infiltration of mature plasma cells, plasmacytoid cells, and small lymphocytes (Fig. 1c). Scattered Russell bodies (intracytoplasmic inclusions) and a few immature plasma cells, large transformed lymphocytes, including immunoblasts and histiocytes, were present in all three cases (Fig. 1c), but there were no Dutcher bodies (intranuclear inclusions), centrocyte-like (CCL) cells, or amyloid deposition. There was no remarkable eosinophilic infiltration in any of the three lesions. Three lesions contained scattered multinucleated giant cells. At the boundaries of the nodules, the inflammatory process extended into the adjacent parenchyma, showing fibrous endings of the alveolar septa with lymphoplasmacytic infiltration (interstitial pneumonia pattern) in all three cases (Fig. 1b). EVG staining demonstrated prominent obliterative phlebitis and arteritis in one case (no. 3) (Fig. 1d). However, there was no necrotic area in Case 3.

Staining for CD20, CD3, and CD5 showed the mixed nature of the small lymphocytes. The majority of large transformed lymphocytes, including immunoblasts, expressed B-cell antigen. Immunohistochemical studies of light chain determinants for plasma cells, plasmacytoid cells, and B-immunoblasts have demonstrated a polyclonal pattern (Figs. 1e and f). There were numerous IgG-positive plasma cells with scattered IgA- or IgM-positive plasma cells. However, IgG4-positive cells comprised only 5–10% of the IgG-positive plasma cells. There were no $CD20^+$, $CD5^+$, $CD43^+$, or cyclin $D1^+$ medium-sized lymphocytes in any of the three lesions. Staining with monoclonal antibody, a cocktail of CD21 and CD35 and CD23 highlighted the meshwork of follicular dendritic cells (FDCs). The FDC networks usually showed a normal/reactive pattern. There were no lymphoepithelial lesions

**Table 1**
Summary of clinical findings.

| | Age/ gender | Symptom | Location (number) | Size (cm) | Hilar LA | Autoantibody | IgG4 (mg/ dl) | Treatment | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 58/M | Bloody sputum | Right lower lobe, peripheral (1) | 4 | – | NE | 18.1 | Video-associated thoracoscopic surgery | 4 m alive (–) |
| 2 | 68/M | – | Bilateral lobe, peripheral (4) | 7 | + | ANA | NE | Video-associated thoracoscopic surgery | 56m alive (+) |
| 3 | 72/M | – | Left lower lobe (1) | 2.5 | + | NE | 52 | Partial lobectomy | 4 m alive (–) |

Abbreviations: LA, lymphadenopathy; NE, not examined; ANA, antinuclear antibody; m, months;(—), without disease; (+), with disease.
Normal range of IgG4 < 135 mg/dl (Ref [16]).