CASE REPORT

A Living Case of Pulmonary Ossification Associated with Osteoclast Formation from Alveolar Macrophage in the Presence of T-cell Cytokines

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Abstract

A 61-year-old woman had been coughing up blood-tinged sputum since May 1998. Chest radiography and computed tomography (CT) scans revealed a solitary mass (3 cm in greatest dimension) in the right lower field, accompanied by a surrounding area of ground glass and reticular appearance. Surgical lung biopsy was performed to the surrounding area. The pathological diagnosis was pulmonary ossification of the dendriform type. Alveolar macrophages obtained from her lung differentiated into tartrate-resistant acid phosphatase (TRAP)-positive multinucleated giant cells (MGCs) in the presence of autologous T cells or of macrophage colony stimulating factor (M-CSF) and interleukin-4 (IL-4). This suggests the possibility that monocytes/macrophages may have the ability to form osteoclasts in the presence of cytokines that may be involved in the development of pulmonary ossification.


Key words: pulmonary ossification, a living case, T-cell cytokines, M-CSF, IL-4

Introduction

Ossification in the lung may occur in association with a variety of diseases. Independently of ossification associated with another primary disease, another form of benign ossification in the lung has been reported; it is known as idiopathic pulmonary ossification. Generally, there are two types of pulmonary ossification, dendriform type and nodular type, and both are composed of mature lamellar bone. Pulmonary ossification has usually been found at autopsy, because the disease is very rare and usually occurs without symptoms. Its pathogenesis is not fully understood. Here we report a living patient with an antemortem diagnosis of pulmonary dendriform ossification, and present in vitro data suggesting that osteoclasts derived from pulmonary macrophages may be involved in the development of pulmonary ossification. As to the etiology of pulmonary ossification, we assume that osteoclasts may play an important role. Osteoclasts are necessary for bone remodeling and absorption, and are generated from progenitors of monocytes/macrophages. It is generally accepted that alveolar macrophages and osteoclasts are differentiated from myeloid precursor cells, including monocytes/macrophages (1, 2). Akagawa et al showed that TRAP-positive osteoclast-like MGCs are generated from human monocytes in the presence of M-CSF and IL-4 (3). Therefore, we hypothesized that transformation from pulmonary macrophages to osteoclasts in the lung leads to pulmonary ossification, and consequently evaluated the response of alveolar macrophages obtained from the patient’s BALF in vitro.

Case Report

Patient

A 61-year-old woman with no history of smoking had been coughing up blood-tinged sputum for a month. Her medical history revealed no mitral valve disease, and no chronic inflammatory diseases, but she had diabetes that had been treated with insulin for some years. Physical examination revealed no abnormalities except for fine crackles on the right lung. Hematological and blood chemistry values including serum Ca, P, and PTH, were normal. Chest radiogra-
A Living Case of Pulmonary Ossification

Figure 1. Chest radiography taken in May 1998 reveals a solitary mass about 3 cm in greatest dimension in the right lower field.

Figure 2. Chest CT scan taken in May 1998 reveals a solitary mass 3 cm in greatest dimension in the right lower lobe, accompanied by a surrounding area of ground glass and reticular appearance.

Figure 3. Histopathological findings (HE x4) show fibrosis and mature lamellar bones whose marrow elements contain mature fat.

phy and computed tomography (CT) scans revealed a solitary mass (3 cm in greatest dimension), accompanied by a surrounding area of ground glass and reticular appearance, in the right lower field (Figs. 1, 2). Sputum culture was normal flora and cytology showed no abnormal findings. Transbronchial lung biopsy (TBLB) and bronchial alveolar lavage (BAL) were performed. Histopathological findings showed no evidence of malignancy, with only mild fibrosis and a mild increase of lymphocyte cell counts in BAL fluid (BALF). A solitary mass was subsequently decreasing in size on chest CT, and this mass was considered inflammatory change like organizing pneumonia. But the surrounding area of ground glass and reticular appearance remained; this area was considered interstitial change like interstitial pneumonitis. Finally, transluminal lung biopsy of the surrounding area of ground glass and reticular appearance was performed by video-assisted thoracoscopic surgery (surgical lung biopsy). Pathological findings were fibrosis and mature lamellar bones whose marrow elements had mature fat (Fig. 3). These bones were distributed diffusely, and ranged in size from 100 μm to 6 mm. The pathological diagnosis was pulmonary ossification dendriform type. TBLB was performed bilaterally on another lobe on a separate occasion, but pathological findings were slight fibrosis only, indicating that pulmonary ossification was localized in the right lower lobe. However, we cannot rule out the possibility that the area of ground glass and reticular appearance in the right lower lobe is only an early stage of systemic ossification, because there is a portion of the lesion in which fibrosis may transform into ossification in the future (Fig. 4) and there is fibrosis in the other bilateral lung fields. Other general body surveys, including head and abdominal CT and bone X-P, were almost normal. The blood-tinged sputum improved spontaneously without medication. At three years after discharge from the hospital, her cough and blood-tinged sputum have remained improved.

Materials and Methods

RPMI 1,640 medium (Nissui Seiyaku Co., Ltd., Tokyo, Japan) was supplemented with 3 mg/ml glutamine (Sigma...
Figure 4. Histopathological findings (HE ×40) show the portion in which fibrosis may transform into ossification.

Figure 5. Development in the presence of M-CSF and IL-4 of TRAP-positive multinucleated giant cells from alveolar macrophages obtained from the patient (8 weeks, magnification ×100).

Figure 6. Development by cocultivation with autologous T cells of TRAP-positive multinucleated giant cells from alveolar macrophages obtained from the patient (8 weeks, magnification ×100).

Aldrich Japan K.K, Tokyo, Japan), 100 U/ml penicillin G potassium (Banyu Seiyaku Co., Ltd., Tokyo, Japan), 100 μg/ml streptomycin (Meiji Seika Co., Ltd., Tokyo, Japan), 10% of autoclaved NaHCO₃ and finally 10% heat-inactivated fetal calf serum (FCS: Z. L. Bockneck Laboratories, Inc., Ontario, Canada). FCS and distilled water were shown to contain 3 pg and less than 1 pg of lipopolysaccharide per ml by the Limulus amebocyte lysate test, respectively. Recombinant human M-CSF (2×10⁸ U/mg) was kindly supplied by Morinaga Milk Industry Co., Ltd., Tokyo, Japan. Recombinant human IL-4 (1×10⁷ U/mg) was obtained from Genzyme Corp, Minneapolis, USA. Human alveolar macrophages were obtained from the patient and healthy volunteers (non-smokers without pathogenesis) by bronchoalveolar lavage. The patient and healthy volunteers consented to permit the use of alveolar macrophage in part of this study by informed consent. Alveolar macrophages were incubated in plastic dishes for 1 hour at 37°C in a CO₂ incubator, and non-adherent cells were removed by repeated washing. More than 97% of the recovered cells were judged to be alveolar macrophages based on morphology, nonspecific esterase staining (cells were stained for α-naphthyl butyrate esterase), CD14 positivity and their ability to phagocytize latex particles. Autologeuous T cells were obtained from the non-adherent cells through the nylon wool column. Human alveolar macrophages (2.5×10⁴ per ml in 12 well tissue culture plates) were cultured with M-CSF (10⁴ U/ml) in the presence of IL-4 (10³ U/ml) for 8 weeks or cocultured with autologous T-cells (macrophage: T cell=1 : 20) for 8 weeks. After culture, the cells were fixed and stained with Diff-Quick (Kokusai Siyaku Co., Ltd., Kobe, Japan) and counted on an inverted microscope. Cells with 3 or more nuclei per cell were defined as MGCs. The results are expressed as the mean MGCs counts and standard deviations of triplicate wells. Number of MGCs was counted in 500 total cells at triplicate wells. Before culture, no limited MGC were observed in culture plate. For TRAP activity, cells were fixed and stained using a commercial kit (Sigma Diagnostics, St Louis, MO) according to the manufacturer’s instructions. TRAP-positive cells appeared dark red.
Results

We present evidence that alveolar macrophages can differentiate into TRAP-positive multinucleated giant cells (MGCs) at high frequency in the presence of M-CSF and IL-4 (Fig. 5) (115.5±30.5 MGCs formation/500 cells after 8 weeks), or when cocultured with autologous T cells (Fig. 6) (13.0±11.5 MGCs formation/500 cells after 8 weeks). These MGCs closely resemble osteoclasts in respect to morphological characteristics and TRAP positivity. However, alveolar macrophages from healthy volunteers form MGCs poorly under the same conditions (Fig. 7) (3.0±2.0 MGCs formation/500 cells after 8 weeks). These findings suggest that T cell-derived cytokines have a role in promoting the formation of multinucleated giant cells from alveolar macrophages.

Discussion

Ossification in the lung may occur in pulmonary alveolar microlithiasis, pulmonary amyloidosis, tracheobronchopathia osteochondroplastica or dystrophic ossification, occasionally accompanied by chronic necrosis or slowly growing tumor masses. Independent of these diseases, another form of benign ossification in the lung, first reported in 1859 by Wagner (4), is known as pulmonary ossification. Generally, there are two types of pulmonary ossification, dendriform type and nodular type, and both types are composed of mature lamellar bone. The dendriform type consists of linear and branching bony deposits within the interstitium and alveolar septa, and the ossification contains a narrow element composed of mature fat and hematopoietic cells. The dendriform type is often associated with lung scarring and interstitial fibrosis. Radiographically, the dendriform type manifests as linear shadows or a lacy appearance in the lower fields (5). The dendriform type is sometimes diffuse, but is predominantly localized. The dendriform type is rare; it was found in 16 cases (0.6%) among 2,800 autopsies (6). Most cases of dendriform ossification are accidentally found at autopsy, because of the very low frequency and lack of subjective symptoms during life.

In contrast, the nodular type consists of ossific bodies in the alveolar space, and the ossification does not contain a marrow element. The nodular type is particularly associated with chronic passive congestion, frequently with mitral stenosis and pulmonary congestion (7, 8), and rarely with left atrial myxoma, constrictive pericarditis, aortic valve disease, coronary artery disease, and systemic hypertension. Radiographically, the nodular type manifests as disseminated nodular calcific densities in the lower fields (5).

The present findings suggest that this case has features compatible with the dendriform type of pulmonary ossification with respect of her histopathological findings and clinical manifestation. But since the pulmonary fibrosis observed in the TBLB specimens on another lobe may develop into diffuse ossification in the future, it is impossible to identify whether this pulmonary ossification is only localized in the right lower lobe. The reason why pulmonary ossification is only localized in the right lower lobe may be local inflammatory change which induced the mass-like organizing pneumonia in the right lower field. In this case, the solitary pulmonary ossification was difficult to diagnose by radiographic findings and TBLB. Thoracoscopic surgery (surgical lung biopsy) was an excellent diagnostic modality for identifying this solitary pulmonary ossification.

In the present case, there was no evidence of disturbance of pulmonary circulation, such as occluded blood supply or chronic passive congestion in the pulmonary parenchyma. On the other hand, we present the possibility that alveolar macrophages from the patient’s BALF may differentiate into TRAP-positive MGCs in the presence of M-CSF and IL-4 or when cocultured with autologous T cells. However, alveolar macrophages from healthy volunteers form MGCs poorly under the same conditions, so we assume these different findings may be related to the etiology of pulmonary ossification. In vitro, osteoclast is defined as TRAP-positive MGCs with osteoclast effect as pit formation in mineral bone. Although we did not analyze the osteoclast effect, TRAP-positive MGCs formation from alveolar macrophages in pulmonary ossification revealed the possibility that osteoclasts were supplied with ectopic cells such as alveolar macrophages and were partly involved in the development of pulmonary ossification. Therefore, we suggest T cell-derived cytokines including M-CSF and IL-4 under the local inflammatory change which induced the mass-like organizing pneumonia in the right lower field may have a role in promoting the formation of MGCs like osteoclasts from alveolar macrophages, and these cells may provoke pulmonary...
ossification. These findings suggest the possibility that monocytes/macrophages may have the ability to form osteoclasts in the presence of cytokines that promote osteoclast generation, leading to pulmonary ossification.

**Conclusion**

We present a 61-year-old non-smoker woman with an antemortem diagnosis of dendriform pulmonary ossification, and show the possibility that alveolar macrophages obtained from her lung may differentiate into TRAP-positive MGCs in the presence of autologous T cells or of M-CSF and IL-4. This is the first case report to suggest the possibility that alveolar macrophages may take part in remodeling of trabeculated bone in alveolar spaces in localized dendriform pulmonary ossification of a living patient.

**References**