Autoimmune Pulmonary Alveolar Proteinosis Complicated with Sarcoidosis: the Clinical Course and Serum Levels of Anti-granulocyte-macrophage colony-stimulating Factor Autoantibody

Toru Arai¹, Takahiko Kasai², Kazunori Shimizu¹, Kunimitsu Kawahara³, Kanako Katayama⁵, Chikatoshi Sugimoto¹, Masaki Hirose¹, Hiroyuki Okamoto⁶, Kazunobu Tachibana¹, Masanori Akira¹, and Yoshikazu Inoue¹

Abstract:
Autoimmune pulmonary alveolar proteinosis (APAP) is caused by macrophage dysfunction due to anti-granulocyte-macrophage colony-stimulating factor (GM-CSF) autoantibody. We experienced 2 cases of APAP complicated with sarcoidosis in a 42-year-old woman and a 51-year-old man (age at the sarcoidosis diagnosis). APAP preceded sarcoidosis in the woman, and both diseases were diagnosed simultaneously in the man. Sarcoidosis lesions were observed in the lung, skin, and eyes, and the pathological findings of APAP were not marked at the diagnosis of sarcoidosis in either case. Low-grade positive serum anti-GM-CSF autoantibody was suspected to be correlated with the occurrence of sarcoidosis and resolution of APAP.

Key words: autoimmune pulmonary alveolar proteinosis, sarcoidosis, anti-granulocyte-macrophage colony-stimulating factor autoantibody

(Intern Med 59: 2539-2546, 2020)
(DOI: 10.2169/internalmedicine.3853-19)

Introduction
Pulmonary alveolar proteinosis (PAP) is characterized by phospholipid and surfactant protein (SP) accumulation in the alveolar spaces (1-4). Autoimmune pulmonary alveolar proteinosis (APAP) is caused by macrophage dysfunction due to anti-granulocyte-macrophage colony-stimulating factor (GM-CSF) autoantibody (2-5). APAP is known to trigger complications in various diseases, including collagen vascular and interstitial lung diseases (3). APAP complicated with sarcoidosis was previously reported (6); however, we believe that the pathophysiological link between the two diseases was not clarified sufficiently.

We experienced two such cases and examined the relationship between the serum anti-GM-CSF autoantibody levels and the clinical course.

Case Reports

Subjects
Out of 102 APAP cases diagnosed in the National Hospital Organization (NHO) Kinki-Chuo Chest Medical Center between 2002 and 2017, 2 were complicated with sarcoidosis. We obtained informed consent to conduct anti-GM-CSF
autoantibody measurements. The institutional review board of the Kinki-Chuo Chest Medical Center approved this retrospective study (Approved Number 674).

**The diagnosis of APAP**

PAP was diagnosed by bronchoalveolar lavage (BAL) and/or histological findings from a transbronchial lung biopsy (TBLB) or surgical lung biopsy (SLB) (3, 4). APAP was defined as anti-GM-CSF autoantibody-positive PAP (3, 4).

**Anti-GM-CSF autoantibody measurement**

We measured the anti-GM-CSF autoantibody by an enzyme-linked immunosorbent assay (ELISA), as previously reported, using a cut-off of 0.5 μg/mL (3).

**The diagnosis of sarcoidosis**

Sarcoidosis was diagnosed based on the 2006 Diagnostic Criteria and Guidelines for Sarcoidosis published by the Japanese Society of Sarcoidosis and Other Granulomatous Disorders (JSSOG) (7, 8). In both cases, lung disease was pathologically diagnosed with a BAL/TBLB or SLB. The disease spread outside the lung, and non-necrotizing epithelioid granulomas were histologically observed in both cases.

**Case 1: The diagnosis of APAP preceded that of sarcoidosis**

A 29-year-old non-smoking woman consulted our hospital because of bilateral pulmonary infiltrative shadows. High-resolution computed tomography (HRCT) revealed a “crazy-paving” pattern, suggesting PAP (Fig. 1A). BAL fluid (BALF) was milky, and a cell analysis revealed macrophages at 72.0%, lymphocytes at 27.6%, and neutrophils at 0.4%. TBLB specimens showed periodic acid-Schiff (PAS)-positive proteinaceous material in the alveolar spaces (Fig. 2C). The serum anti-GM-CSF autoantibody level was 102 μg/mL, and the serum Krebs von den Lungen-6 (KL-6), surfactant protein (SP)-D, SP-A, carcinoembryonic antigen (CEA), and cytokeratin fragment 21-1 (CYFRA) levels were elevated (Table 1). She was diagnosed with APAP and treated with GM-CSF inhalation for 6 months at 30 years old. Following treatment, her ground glass opacity (GGO) decreased, and her disease condition was stable for nine years (Fig. 1A-C). The serum anti-GM-CSF autoantibody levels decreased along with a decrease in the serum KL-6 and SP-D levels.

At 40 years of age, the patient’s shortness of breath deteriorated from modified medical research council score
A 51-year-old former-smoking man (19 pack-years) con-
sulted our hospital with shortness of breath and a dry cough. He had been suffering from a dry cough for about two years. HRCT revealed GGO and reticular opacity (Fig. 3A, B), and hilar and mediastinal lymphadenopathy was observed (Fig. 3C). Serum KL-6, SP-D, SP-A, CEA, and CYFRA levels were elevated (Table 2). Interstitial pneumonia was suspected, and he underwent an SLB. Cellular interstitial pneumonia with various-sized epithelioid cell granulomas involving the peribronchiolar and alveolar walls were observed in the SLB specimens, suggesting sarcoidosis
or hypersensitivity pneumonia (HP) (Fig. 4A, B). Fine granular proteinaceous material (Fig. 4C, D) suggesting PAP was also noted in the alveolar spaces. The anti-GM-CSF autoantibody test was positive, with a value of 18.5 μg/mL (Table 2); however, sarcoidosis was predominant. HP was rejected because his lung disease did not improve spontaneously after being admitted to our hospital and anti-bird and anti-Trichosporon asahii antibodies, the most common etiologies of HP in Japan, were negative. In addition, his elbows and knees showed erupted skin, which revealed well-formed non-necrotizing epithelioid granuloma on a biopsy, findings that were compatible with sarcoidosis. He suffered from a visual field defect, and an ophthalmologic inspection revealed uveitis. Infectious organisms were not detected in the BALF or histological specimens. Given these laboratory findings, we diagnosed the patient with APAP complicated with sarcoidosis.

Six months after diagnosis of the two diseases, his FVC decreased while his KL-6, SP-D, and sIL-2R increased, corresponding to a decrease in serum anti-GM-CSF autoantibody levels. The patient complained of shortness of breath and needed oxygen inhalation. His serum markers (ACE, sIL-2R, lysozyme, KL-6, and SP-D) decreased, and his respiratory dysfunction improved following prednisolone administration (30 mg daily).

Discussion

We presented two cases of APAP complicated with sarcoidosis. In one case, sarcoidosis occurred after the remission of APAP, while in the other case, sarcoidosis was the predominant disease, and APAP was diagnosed simultaneously. Of note, the serum anti-GM-CSF autoantibody results were positive in both cases, but the levels were low at the time of the diagnosis of sarcoidosis. We therefore suspect a pathophysiological link between APAP and sarcoidosis.

Sarcoidosis is a systemic granulomatous disease with an unknown etiology that is histologically characterized by well-formed non-necrotizing epithelioid granuloma (9, 10). It commonly affects young and middle-aged adults and shows bilateral hilar lymphadenopathy, pulmonary infiltration, and ocular and cutaneous lesions. Previous investigators have suggested environmental exposure of microbial agents, including mycobacterium and Propionibacterium acnes, as possible causative factors (10). The infiltration of macrophages and T-lymphocytes, as well as various cytokines, including tumor necrosis factor-α and GM-CSF, contributes to granuloma formation (10). Increased GM-CSF messenger ribonucleic acid (mRNA) in the BAL is correlated with the clinical activity of sarcoidosis and lymphocytes in the BAL, while serum ACE levels can be significantly elevated in cases of sarcoidosis with an increased ex-
expression of GM-CSF mRNA (11).

The pathophysiological role of GM-CSF differs between APAP and sarcoidosis. APAP cases are generally immunocompromised owing to dysfunctional macrophages and neutrophils due to the presence of anti-GM-CSF autoantibodies (12, 13). Previous reports have therefore described cases of chronic infection of mycobacterium, fungus, and nocardia (14, 15). Bacteria can grow subclinically in APAP patients before apparent infectious foci occur in the body with clinical symptoms. For example, *Mycobacterium avium* (MAC) was detected in fluids recovered from the lung washed by whole-lung lavage (16), despite no apparent radiological findings indicative of MAC infection being detected on HRCT films of APAP. Therefore, the causative microbial agents of sarcoidosis may accumulate in the lungs of APAP patients before the clinical presentation.

In Case 1, sarcoidosis occurred following remission of APAP. In Case 2, disease conditions associated with sarcoidosis worsened after the simultaneous diagnosis of sarcoidosis and APAP. In both cases, the serum anti-GM-CSF autoantibody levels continuously decreased, and functionally normalized macrophages may have responded to the increased causative microbial agents and either induced or aggravated sarcoidosis. Trapnell et al. suggested the critical threshold of serum anti-GM-CSF autoantibody level for determining a normal macrophage function to be around 10 μg/mL (17, 18). In both of our cases, prednisolone treatment was needed to control sarcoidosis when the anti-GM-CSF autoantibody levels dropped below 10 μg/mL. Boerner et al. also reported an APAP case complicated with sarcoidosis (Table 3, Case A), in which sarcoidosis occurred after the remission of APAP treated with whole-lung lavage (6). It is possible that the normalized macrophage function induced sarcoidosis in the patient (Case A). Hoffman et al. reported that the alveolar macrophage function of PAP cases improved after the whole-lung lavage (19); however, serial levels of serum anti-GM-CSF autoantibody were not measured in this study.

There are a few reports supporting our hypothesis that some infection-related diseases, including sarcoidosis, occur following APAP remission. Our institution reported a case of tuberculous lymphadenitis that occurred after remission of APAP treated with GM-CSF inhalation therapy (20). The serum anti-GM-CSF autoantibody levels of that case at the time of the tuberculous lymphadenitis diagnosis were also about 10 μg/mL (unpublished data). Possible causative pathogens of sarcoidosis, including mycobacterium and *P. acnes*, were not detected by culture in the biopsy specimens of the two cases presented in our manuscript. However, immune-staining using PAB antibody (21) might be able to detect *P. acnes* in biopsy specimens, although we did not perform that kind of investigation for our two cases.

Granulomas are generally formed to confine pathogens, restrict inflammation, and protect surrounding tissues (10). In APAP cases, the pathogen can easily enter the lymphatic system without being contained in a granuloma at the initial infected site, as the granuloma may not be sufficiently formed. Therefore, a causative pathogen contracted via the airway can easily spread to systemic organs through the bloodstream. The presence of cutaneous and ocular lesions associated with sarcoidosis in both of our cases was consistent with the hypothesis that macrophage dysfunction observed in two previous APAP cases caused the systemic spread of microbes that were potentially causative for sarcoidosis.

Cutaneous and pulmonary diseases improved in both

---

**Table 3. Reported Cases of APAP Complicated with Sarcoidosis and Our Cases.**

|                | Case A | Case B | Case C | Case 1 | Case 2 |
|----------------|--------|--------|--------|--------|--------|
| Gender         | Female | Female | Female | Female | Male   |
| Age at APAP diagnosis, years | 57     | 65-66  | 58     | 29     | 51     |
| Age at sarcoidosis diagnosis, years | 58-59  | 64     | 51     | 40     | 51     |
| Other conditions | No     | Scleroderma | No     | ANA (Nucleolar) | Anti-MAC Ab(+) |
| Preceding disease | APAP  | Sarcoioid | Sarcoioid | APAP  | Simultaneous |
| Trigger of APAP | No     | Steroid | No     | No     | No     |
| Trigger of sarcoidosis | WLL    | No     | No     | No     | No     |
| Serum levels of anti-GM-CSF Ab at sarcoidosis diagnosis, μg/mL | NE     | 35.1   | NE     | 3.53   | 18.5 (6.2*) |
| Serum levels of anti-GM-CSF Ab at APAP Diagnosis, μg/mL | NE     | 10.8   | 4.8    | 102    | 18.5   |
| Steroid use at onset of APAP | No     | Yes    | No     | No     | No     |
| Steroid for respiratory failure due to sarcoidosis | (-)    | (+)    | (-)    | (+)    | (+)    |
| Organs of sarcoidosis | Lung, hilar LN | Lung, hilar LN, eye, liver, muscle | Lung, hilar LN | Lung, hilar LN, eye, skin | Lung, hilar LN, eye, skin |

APAP: autoimmune pulmonary alveolar proteinosis, ANA: anti-nuclear antibody, MAC: mycobacterium avium complex, WLL: whole lung lavage, GM-CSF: granulocyte-macrophage-colony-stimulating factor, LN: lymph nodes

*: Anti-GM-CSF antibody decreased at the start of corticosteroid when disease activity of sarcoidosis of Case 2 was worsened.

**: Case 1 and Case 2 were reported in this manuscript.
cases following treatment with corticosteroids. Akasaka et al. reported that corticosteroid administration induced aggrava-
tion of APAP activity because corticosteroids suppressed
the function of alveolar macrophages in addition to anti-
GM-CSF autoantibodies in the blood (22). Indeed, Yamasue
et al. reported a preceding case of sarcoidosis in which PAP
occurred after introducing steroid therapy (Table 3, Case
B) (23). Anti-GM-CSF autoantibodies were retrospectively
detected in preserved serum material collected before steroid
therapy (23). We have reduced the corticosteroid dose as
much as possible for our two cases and are cautiously ob-
serving the disease activity of sarcoidosis in order to prevent
APAP recurrence.

Three previously reported cases (6, 23, 24) and our two
present cases of APAP complicated with sarcoidosis were
reviewed in Table 3. Sarcoidosis preceded APAP in two
cases (Case A, Case 1), APAP preceded sarcoidosis in two
cases (Case B, C), and both diseases were simultaneously
diagnosed in one case (Case 2). The pathophysiology of sarco-
dosis preceding APAP (Case B, C) might differ from that of
APAP preceding sarcoidosis. In Case B and C, chronic
inflammation of sarcoidosis may have been associated with
the induction or upregulation of anti-GM-CSF antibody lev-
els, leading to APAP. Immunosuppressive therapy for sarco-
dosis might also have affected the occurrence of APAP in
Case B. Regarding Case B, anti-GM-CSF antibody was positive at the sarcoidosis diagnosis, so APAP might have
occurred insidiously before the sarcoidosis diagnosis and
then reoccurred after the immunosuppressive therapy (25).
From the standpoint of this hypothesis, the multorgan in-
volvement of sarcoidosis observed in Case B is thus consid-
ered to be consistent with our cases.

In conclusion, we experienced two cases of APAP compli-
cated with sarcoidosis. Low-grade positive serum anti-GM-
CSF autoantibody was suspected to be correlated with the
occurrence of sarcoidosis and resolution of APAP. Further
studies are needed to draw definite conclusions concerning
the pathophysiologic link between APAP and sarcoidosis.

Author’s disclosure of potential Conflicts of Interest (COI).
Yoshikazu Inoue: Advisory role, Boehringer Ingelheim.

Financial Support
This study was partially supported by a grant from the Na-
tional Hospital Organization {H28-NHO (Kokyu)-2} awarded to
T.A. and Y.I.; AMED, DLD/14526278 awarded to Y.I.; and PAP/
14526182 awarded to T.A. and Y.I.

Acknowledgement
We are grateful to Akiko Matsumuro for measuring the anti-
GM-CSF autoantibody levels.

References
1. Roasen SH, Castleman B, Liebow AA. Pulmonary alveolar protei-
nosis. N Engl J Med 258: 1123-1142, 1958.
2. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteino-
sis. New Engl J Med 349: 2527-2539, 2003.
3. Inoue Y, Trapnell BC, Tazawa R, et al.; Japanese Center of the
Rare Lung Diseases Consortium. Characteristics of a large cohort
of patients with autoimmune pulmonary alveolar proteinosis in Ja-
pan. Am J Respir Crit Care Med 177: 752-762, 2008.
4. Kumar A, Abdelmalak B, Inoue Y, Culver DA. Pulmonary alveolar
proteinosis in adults: pathophysiology and clinical approach. Lan-
cet Respir Med 6: 554-565, 2018.
5. Kitamura T, Uchida K, Tanaka N, et al. Serological diagnosis of
idiopathic pulmonary alveolar proteinosis. Am J Respir Crit Care
Med 162: 658-662, 2000.
6. Boerner EB, Costabel U, Wessendorf TE, et al. Pulmonary alveo-
lar proteinosis: another autoimmune disease associated with sarcoi-
dosis. Sarcoid Vasc Diffuse Lung Dis 33: 90-94, 2016.
7. Sawahata M, Sugiyama Y, Nakamura Y, et al. Age-related and his-
torical changes in the clinical characteristics of sarcoidosis in Ja-
pan. Respir Med 109: 272-278, 2015.
8. The Japanese society of sarcoidosis and other granulomatous dis-
orders (JSSOG) diagnostic criteria and guidelines for sarcoidosis.-
2006. Jpn J Sarcoidosis Other Granulomatous Disease 22: 89-101,
2007.
9. The Joint statement of the ATS/ERS/WASOG was adopted by the
ATS Board and by the ERS Executive Committee. Statement of
Sarcoidosis. Am J Respir Crit Care Med 160: 736-755, 1999.
10. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J
Med 357: 2153-2165, 2007.
11. Kuzumaki N. Correlation of GM-CSF mRNA in bronchoalveolar
fluid with indices of clinical activity in sarcoidosis. Thorax 48:
1230-1234, 1993.
12. Uchida K, Nakata K, Trapnell BC, et al. High-affinity autoanti-
bodies specifically eliminate granulocyte-macrophage colony-
stimulating factor activity in the lungs of patients with idiopathic
pulmonary alveolar proteinosis. Blood 103: 10879-11098, 2004.
13. Uchida K, Beck DC, Yamamoto T, et al. GM-CSF autoantibodies
and neutrophils dysfunction in pulmonary alveolar proteinosis. N
Engl J Med 356: 567-579, 2007.
14. Punatar AD, Kusne S, Blair JE, Seville MT, Vikram HR. Opportu-
nistic infections in patients with pulmonary alveolar proteinosis.
J Infection 65: 173-179, 2012.
15. Seymour JF, Presnell JJ. Pulmonary alveolar proteinosis. Am J
Respir Crit Care Med 166: 215-235, 2002.
16. Witty LA, Tapson VF, Piantadosi CA. Isolation of mycobacteria in
patients with pulmonary alveolar proteinosis. Medicine 72:
103-109, 1994.
17. Uchida K, Nakata K, Carey B, et al. Standardized serum GM-CSF
autoantibody testing for the routine clinical diagnosis of autoim-
mune pulmonary alveolar proteinosis. J Immunol Methods 402:
57-70, 2014.
18. Trapnell BC, Carey BC, Uchida K, Suzuki T. Pulmonary alveolar
proteinosis, a primary immunodeficiency of impaired GM-CSF
stimulation of macrophages. Curr Opin Immunol 21: 514-521,
2009.
19. Hoffman RM, Dauber JK, Rogers RM. Improvement in alveolar
macrophage migration after therapeutic whole lung lavage in pul-
monary alveolar proteinosis. Am Rev Respir Dis 139: 1030-1032,
1989.
20. Nakamura Y, Matsumura A, Katsura H, Sakaguchi M, Ito U, Nita-
iich M. A case report: mediastinal tuberculosis lymphadenitis
complicated with pulmonary alveolar proteinosis. J Japanese Asso-
ciation for Chest Surgery 23: 45-48, 2009.
21. Negi M, Takemura T, Guzman J, et al. Localization of Propioni-
bacterium acnes in granulomas supports a possible etiologic link
between sarcoidosis and the bacterium. Mod Pathol 25: 1284-
1297, 2012.
22. Akasaka K, Tanaka T, Kitamura N, et al. Outcome of corticoster-
oid administration in autoimmune pulmonary alveolar proteinosis;
23. Yamasue M, Nureki S, Usagawa Y, et al. Elevated serum anti-GM-CSF antibodies before the onset of autoimmune pulmonary alveolar proteinosis in a patient with sarcoidosis and systemic sclerosis. Tohoku J Med 243: 77-83, 2017.

24. Tanaka Y, Shirai T, Asada K, Muramatsu A, Katsumata M, Suda T. Autoimmune pulmonary alveolar proteinosis in a patient with sarcoidosis. Clin Case Rep 7: 731-734, 2019.

25. Imura Y, Yukawa N, Handa T, et al. Two cases of autoimmune and secondary pulmonary alveolar proteinosis during immunosuppressive therapy in dermatomyositis with interstitial lung disease. Mod Rheumatol 28: 724-729, 2018.

The Internal Medicine is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (https://creativecommons.org/licenses/by-nc-nd/4.0/).