

TEMPORARY REMISSION OF AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS AFTER INFECTIOUS EPISODES

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ABSTRACT. Pulmonary alveolar proteinosis (PAP) is a rare disease of unknown aetiology. Although resolution occurs in about 30% of autoimmune PAP (APAP) cases, its pathogenesis is not yet sufficiently understood. Two APAP cases at our institute showed remission following infectious episodes. Case 1: a 40-year-old female APAP patient suffered from herpes encephalitis and was treated with an antiviral drug. Her symptoms and radiological results resolved within two months of her recovery from the encephalitis. Case 2: A 53-year-old male current-smoker APAP patient was admitted for pneumonia. After treatment with antibiotics, his radiological results and symptoms improved. He experienced a similar resolution of APAP after another infectious episode two years later. Remission of APAP may occur following viral or bacterial infection. We hypothesise that remission of APAP is triggered by the induction of granulocyte-macrophage colony-stimulating factor (GM-CSF) following viral or bacterial infection. Further studies of APAP remission, and especially of the effects of GM-CSF induction, are needed. (*Sarcoidosis Vasc Diffuse Lung Dis* 2017; 34: 85-90)

KEY WORDS: pulmonary alveolar proteinosis, infection, remission

INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare disease of unknown aetiology, characterized by the accumulation of intraalveolar proteinaceous material that is rich in lipids and tests positive using the periodic acid-Schiff (PAS) stain (1). It has been shown that most idiopathic PAP patients have neutraliz-

ing autoantibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF). These cases are considered to be autoimmune PAP (APAP) (2). The pathophysiology of APAP is thought to be macrophage dysfunction caused by the neutralizing antibodies. The natural history of PAP can follow one of three pathways: spontaneous remission, stabilisation with persistent symptoms, or progressive deterioration (3). Spontaneous resolution occurs in about 30% of APAP cases (4). Some PAP cases partially improve following treatment of bacterial infections (5). However, the pathogenesis is uncertain and the course of APAP is unpredictable, with periodic flare-ups alternating with remission. Here we describe two APAP cases that showed remission in the disease severity, focusing on the relationship between the clinical course of infectious episodes and serum parameters.

Received: 31 December 2015

Accepted after revision: 25 January 2017

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CASE PRESENTATION

Case 1

A 40-year-old female who never smoked was referred to our hospital with complaints of chronic cough and shortness of breath (Grade 1 of the modified Medical Research Council (MRC) scale (6)) over six months. Her physical examination revealed neither rales nor clubbed fingers. Her blood test results were as follows: white blood cell (WBC) count of $6.2 \times 10^9/L$ (63.6% neutrophils, 27.3% lymphocytes, 7.8% monocytes and 1.3% eosinophils); haemoglobin of 14.8 g/dL; platelet count of $220 \times 10^9/L$; and serum lactate dehydrogenase (LDH) of 211 U/L. Her serum biomarkers for APAP (4) were elevated to high levels: she had carcinoembryonic antigen (CEA) levels of 18.1 ng/mL; cytokeratin fragment (CYFRA) 21-1 levels of 4.1 ng/mL; Krebs von den Lungen 6 (KL-6) of 17000 U/mL; surfactant protein (SP)-D levels of 168.0 ng/mL; and SP-A levels of 116.0 ng/mL (the normal cut-off levels for each biomarker were 5.0 ng/mL, 2.6 ng/mL, 500 U/mL, 110 ng/mL and 30 ng/mL, respectively). The chest radiograph showed bilateral infiltrative shadows, and a high-resolution computed tomography (HRCT) scan confirmed widespread, bilateral ground-glass attenuation (GGA) with thickened interlobular and intralobular septa, generally referred to as a 'crazy paving' pattern. The bronchoalveolar lavage fluid (BALF) was milky in appearance, and cytological evaluation showed that micronodular materials and large foamy macrophages were prominent. Anti-GM-CSF autoantibodies were detected in her serum (80.6 g/mL). We therefore diagnosed her condition as APAP.

The patient's arterial oxygen tension (PaO_2) under room air was 71.4 Torr. Her pulmonary function was severely disturbed, and her forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLco) were 58% and 42.7% of her predicted values, respectively. Her pulmonary symptoms did not improve spontaneously, and she was treated with GM-CSF inhalation from April to October 2007, according to the protocol of the phase II trial of GM-CSF inhalation in Japan (7). Thereafter, a whole lung lavage (WLL) was performed in April 2009, and a bronchoscopic lung lavage (left B¹⁺² and left B₈) in October 2010. After the treatment by one segmental lavage with bronchoscopy in 2010, her pulse oxime-

try (SpO_2) has been stable around 92%- 94% (room ambient), and no additional treatment had been needed from October 2010 to April 2013. The findings of chest X-ray during follow-up had been stable.

In April 2013, this patient had a high fever without worsening pulmonary symptoms. She developed refractory seizures and was diagnosed as having encephalitis associated with herpes simplex virus (HSV), based on a computed tomography (CT) head scan and the detection of viral deoxyribonucleic acid in her cerebrospinal fluid (CSF) via polymerase chain reaction. She was transferred to a neurosurgical hospital and received antiviral treatment. Along with remission in her exertional dyspnoea, her SpO_2 levels improved from 96% to 98%, serum levels of KL-6 decreased from 9837 U/mL to 4819 U/mL, and findings of her chest X-ray resolved. Her serum anti-GM-CSF autoantibody levels also decreased from 51.3 pg/mL to 31.0 pg/mL after this infectious episode (Table 1, Figure 1).

Case 2

A 53-year-old man was referred to our hospital in June 2005 with complaints of shortness of breath (modified MRC Grade 1 (6)). He was a current smoker who had smoked one pack per day from the age of 20. HRCT scans revealed subpleural heterogeneous GGA, including partial consolidations. His blood test results were: WBC count of $3.5 \times 10^9/L$ (51.1% neutrophils, 38.1% lymphocytes, 8.1% monocytes, and 1.0% eosinophils); haemoglobin of 14.9 g/dL; platelet count of $148 \times 10^9/L$; and LDH of 147 U/mL. His serum biomarkers were elevated: he had a CEA of 11.4 ng/mL; CYFRA 21-1 of 11.1 ng/mL; KL-6 of 5050 U/mL; SP-D of 184 ng/mL; and SP-A of 95.7 ng/mL. Arterial blood gas analysis revealed that his PaO_2 under room air was 67.8 Torr in a supine position. His BALF was milky in appearance, and transbronchial lung biopsy specimens showed that the alveoli with preserved lung architecture were filled with PAS-positive microgranular materials. He was diagnosed as having APAP based on a positive test result for anti-GM-CSF antibodies in his serum (204.31 g/mL). The patient was treated with GM-CSF inhalation (7) from December 2004 until March 2005. His alveolar-arterial oxygen difference decreased by more than 10 Torr and his pulmonary symptoms improved slightly. His SpO_2

Table 1. Laboratory results before and after the herpes encephalitis (Case 1)

	Apr. 2012 1 y before HE	Jan. 2013 3 mo before HE	Apr. 2013 HE	2 d after HE	Apr. 2014 1 y after HE
WBC (x100/ L)	53	56	60	129	58
CRP (mg/dL)	0.1	0.1	0.1	0.2	0.3
CEA (ng/dL)	18.8	19.3	15.1	14.8	11.3
CYFRA (ng/dL)	5.9	4.8	4.8	2.8	3
KL-6 (U/mL)	12952	13907	10814	9837	6582
SP-D (ng/mL)	375	278	267	60.5	188
Anti-GM-CSF Ab (g/mL)	48.9	98.6	51.3	37	N.A
GM-CSF (pg/mL)	1.7	N.A	2.7	2.8	2.2

HE: herpes encephalitis; WBC: white blood cell count; CRP: C-reactive protein; CEA: carcinoembryonic antigen; KL-6: Krebs von den Lungen-6; SP-D: surfactant protein-D; Anti-GM-CSF Ab: anti-granulocyte-macrophage colony-stimulating factor autoantibody; GM-CSF: granulocyte-macrophage colony-stimulating factor; mo: month; y: year; d: day; N.A: not available

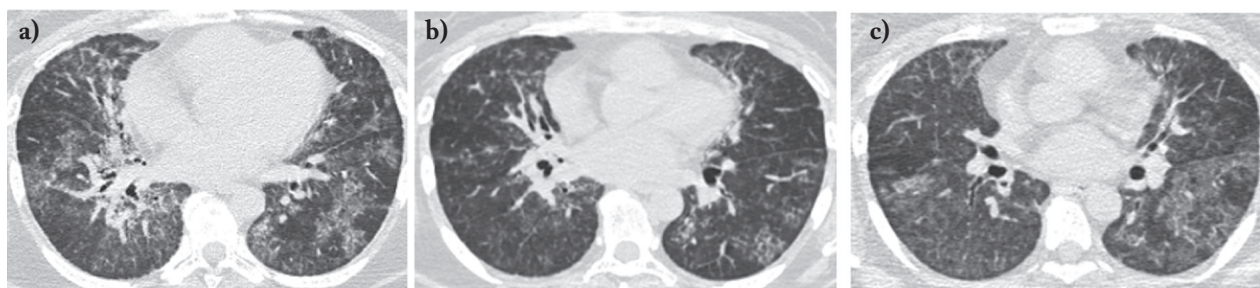


Fig. 1. Serial change in high-resolution computed tomography (HRCT) results before and after an episode of herpes simplex virus (HSV)-associated encephalitis (Case 1). (a) Chest HRCT taken three months before the HSV-associated encephalitis. Bilateral ground-glass attenuation (GGA) associated with thickened interlobular septa and characteristics of autoimmune pulmonary alveolar proteinosis can be seen. HRCT findings improved at diagnosis of HSV-associated encephalitis (b) and two months after the infectious episode (c)

improved from 94% to 96% over the course of this treatment, and his pulmonary symptoms stabilised for several years.

In January 2010, he was complaining of fever and a productive cough and was re-admitted to hospital. The productive cough worsened and was complicated by pneumonia. We did not perform BAL because we were afraid the lung disease of the patient might be worsened by the bronchoscopy procedure. Although we performed the sputum examination, we could not identify the pathogen of the disease. He received antibiotics empirically (ciprofloxacin, 200 mg, twice per day), and his cough improved in two weeks. HRCT scans showed that the GGA on both sides of his lungs, which suggested PAP, improved with respect to peripheral shadows after the treatment. Additionally, his serum levels of KL-6 decreased from 3980 U/mL to 2440 U/mL (Table 2, Figure 2). His SpO₂ levels (93% to 97%) and serum biomarker levels indicating APAP returned four months after the pneumonia episode. He had a similar infectious episode in February 2012 when he was complaining

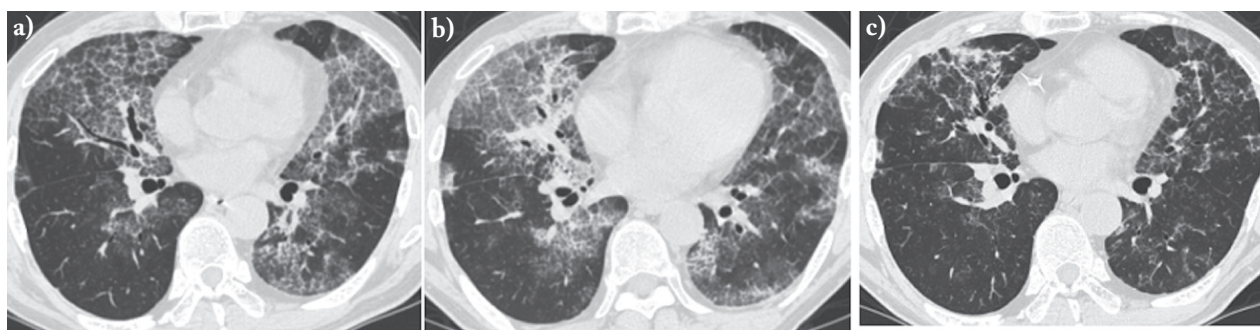
of a worsening productive cough. He received antibiotics (ciprofloxacin, 200 mg, twice per day) for two weeks, and his symptoms improved, along with his radiological results (Table 3, Figure 3). Although his SpO₂ levels (95%) did not change, his serum levels of KL-6 returned from 1097 U/mL to 1560 U/mL three months after the pneumonia episode.

Analysis of serum GM-CSF and anti-GM-CSF autoantibodies

We examined the relationship between serum levels of GM-CSF and anti-GM-CSF autoantibodies, retrospectively. We measured serum levels of free anti-GM-CSF autoantibodies, i.e. those not forming an immunocomplex with GM-CSF, using an enzyme-linked immunosorbent assay (ELISA), as previously reported (the detection limit of anti-GM-CSF autoantibodies: 0.5 g/mL) (4,8). Serum GM-CSF was measured using a commercially available ELISA kit for GM-CSF (R&D SYSTEMS, Metropolis in USA) (Tables 1-3). Serum GM-CSF

Table 2. Laboratory results before and after the pneumonia (PN) (Case 2, episode 1). Abbreviations as for Table 1

	Apr 2009 9 mo before PN	Dec 2009 2 mo before PN	Jan 2010 PN	Mar 2010 2 mo after PN	May 2010 4 mo after PN
WBC (x100/ L)	37	48	83	47	75
CRP (mg/dL)	0.08	0.4	0.06	0.04	0.01
CEA (ng/dL)	8	10.1	12.6	7.5	N.A
CYFRA (ng/dL)	2.5	6.7	8.2	3.5	1.6
KL-6 (U/mL)	727	3110	3980	2440	N.A
SP-D (ng/mL)	57	225	81	93	N.A
Anti-GM-CSF Ab (g/mL)	85.1	64	66.3	N.A	43.2
GM-CSF (pg/mL)	N.A	2.5	2	3.8	N.A

**Fig. 2.** Serial changes in HRCT results before and after the first episode of pneumonia (Case 2, episode 1). Chest HRCT showed bilateral GGA and consolidations two months before the pneumonia episode (a). At admission the patient was diagnosed with pneumonia complications (b) and received antibiotics. The GGA and consolidations improved one month after the pneumonia episode (c)**Table 3.** Laboratory results before and after the pneumonia (Case 2, episode 2). Abbreviations as for Table 1

	Feb 2011 1 y before PN	Aug 2011 6 mo before PN	Dec 2011 3 mo before PN	Feb 2012 PN	May 2012 3 mo after PN	Nov 2012 9 mo after PN
WBC (x100/ L)	35	31	41	5	37	55
CRP (mg/dL)	0.05	0.02	0.06	14.19	0.04	0.26
CEA (ng/dL)	6.8	7.7	7.1	N.A	8	8.1
CYFRA (ng/dL)	5.9	3.1	5	N.A	4.9	5.1
KL-6 (U/mL)	3180	1600	1230	1097	1560	2538
SP-D (ng/mL)	99	95	97	28	75.9	68.2
Anti-GM-CSF Ab (g/mL)	N.A	51.9	N.A	78	N.A	30.6
GM-CSF (pg/mL)	3.8	N.A	3.9	2.2	N.A	1.8

levels in all three episodes were below the normal cut-off levels (7.8 pg/mL). Serum levels of anti-GM-CSF autoantibodies decreased after the diagnosis of the infections.

DISCUSSION

There are two aspects to the association between APAP and infection. On one hand, the dysfunction of alveolar macrophages and neutrophils observed in APAP cases is thought to increase the risk of an op-

portunistic infection (9), including those by *Nocardia* (5), *Mycobacterium* (10), and *Aspergillus* (11) species. On the other hand, infections might affect the onset and progression of APAP (12-14). Indeed, infection might retard APAP, because remission of PAP after the treatment of aspergillosis has been observed (13). Based on the clinical courses by the precise observation of these cases, we believed the infectious episodes might be the triggers of remission of APAP. In case 1, three years from the previous WLL to the resolution of APAP after HSV infection were too long to consider the WLL improved APAP.

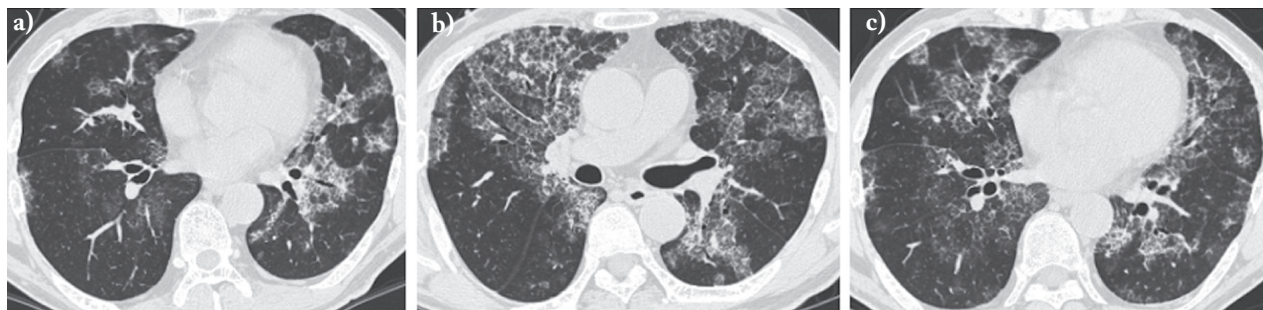


Fig. 3. Serial changes in HRCT results before and after the second episode of pneumonia (Case 2, episode 2). This infectious episode was similar to the previous one. HRCT scans shown are from five months before the pneumonia episode (a), at diagnosis of pneumonia (b) and two weeks after the pneumonia episode (c)

Whether infectious episodes improve or aggravate APAP activity might depend on the species of pathogenic organisms. With respect to *Aspergillus* infections, supernatant from aspergilli cultures has been shown to suppress macrophage function (15,16). Aspergilli stimulate SP-D production by type II alveolar epithelial cells *in vitro* (17). An *Aspergillus* infection might therefore cause a deterioration of APAP symptoms via these effects. The *Mycobacterium tuberculosis* 19-kDa lipoprotein inhibits expression of the Fc γ receptor (18). The elevation of serum levels of anti-GM-CSF autoantibodies caused by suppression of the internalisation of anti-GM-CSF autoantibodies via the receptor might lead to the deterioration of APAP when it is complicated with tuberculosis.

We speculated the remission of APAP after the infections described in our case studies might be triggered by GM-CSF induction following viral or bacterial infection. Serum GM-CSF was elevated in patients with community-acquired pneumonia, including viral infection and unidentified pathogens (19). We did not directly evaluate the alveolar macrophage function, but have shown that the serum levels of GM-CSF were not elevated in these two cases, and that the serum levels of anti-GM-CSF autoantibodies might have decreased following the infectious episodes. Uchida et al. reported that serum GM-CSF in APAP is more abundant than previously reported, and that it exists in a form of an immunocomplex (20). The GM-CSF, induced by infection, and anti-GM-CSF autoantibodies might have formed an immunocomplex in our two cases. As a result, the serum levels of free GM-CSF, i.e. that not forming an immunocomplex, were below cut-off lev-

els at the onset of infection. Concentration of both types of GM-CSF, i.e. the free and immunocomplex forms, might have increased at the onset of infection, although measurement of the immunocomplex-forming GM-CSF is unfortunately impossible.

HSV encephalitis causes elevation of the levels of cytokines such as interleukin 6 (IL-6) and interferon- γ in the CSF. Moreover, a previous report suggests that production of serum GM-CSF leads to anti-HSV-1 immunity against the trans-neuronal spread of challenged HSV-1 within the visual system in animal models (21). The GM-CSF that is released from the CSF into the blood might influence the alleviation of APAP.

Previous reports have suggested a potential role for GM-CSF in the treatment of PAP (22-25). Remission in pulmonary symptoms following treatment with GM-CSF further reinforces the speculation that GM-CSF production is important in the pathogenesis of the remission of APAP following infectious episodes. We understand that we can't definitely conclude from only two cases.

In conclusion, these two case studies suggest that remission of APAP might be triggered by GM-CSF induction following viral or bacterial infection. It is therefore necessary to further investigate the role of GM-CSF and anti-GM-CSF autoantibodies using more cases involving APAP patients.

ACKNOWLEDGEMENTS

We acknowledge Koh Nakata (Niigata University Medical and Dental Hospital, Niigata, Japan) for technical support with the ELISA of anti-GM-CSF autoantibodies.

This study is supported by the grants of Practical Research Project for Rare/Intractable Disease of the Japan Agency for Medical Research and Development, AMED 15652554 (Y.I., T.A., A.M., M.K.), the grants from the Diffuse Lung Diseases Research Group from the Ministry of Health, Labour and Welfare (Y.I.), and the grants from the National Hospital Organization, Japan (Y.I., T.A.).

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