

Implication of Immunohistochemistry for *Propionibacterium acnes* in Differential Diagnosis of Necrotizing Granuloma

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Abstract

Propionibacterium acnes (*P. acnes*) have been reported to have an etiologic link with sarcoidosis. A 45-year-old Japanese woman complaining of cough for 1 month presented to our hospital. Chest computed tomography showed an irregular nodular shadow in the right upper pulmonary lobe, a finding suggestive of either pulmonary sarcoidosis or tuberculosis. Biopsy specimens from the pulmonary shadow showed necrotizing granulomas, and there were no other findings from the initial laboratory examinations that could provide a definitive diagnosis. However, immunohistochemical staining using a *P. acnes*-specific monoclonal antibody revealed small round bodies within the granulomas. Based on these results, we diagnosed the patient clinically with sarcoidosis, and orally inhaled ciclesonide was administered. At a 7-month follow up, the patient had improved clinically and radiologically. The outcome of this case indicates that immunohistochemical evaluation using a *P. acnes* antibody may be useful for diagnosing necrotizing granuloma.

Keywords: Necrotizing granuloma; *Propionibacterium acnes*; Sarcoidosis; Tuberculosis

Abbreviations: ACE: Angiotensin-Converting Enzyme; ANCA: Antineutrophil Cytoplasmic Antibodies; BALF: Bronchoalveolar Lavage Fluid; CT: Computed Tomography; NSG: Necrotizing Sarcoid Granuloma; PAB Antibody: *Propionibacterium acnes*-Specific Monoclonal Antibody that Reacts with Cell-Membrane-Bound Lipoteichoic Acid; *P. acnes*: *Propionibacterium acnes*; TBLB: Transbronchial Lung Biopsy

Introduction

Important causes of granulomatous lung disease in Japan include sarcoidosis and tuberculosis, which are difficult to differentiate. The two diseases are not so rare in Japan, where 7.5 to 9.3 individuals per 100,000 people are estimated to have sarcoidosis, and 16.1 per 100,000 people are estimated to have tuberculosis [1]. Distinguishing pulmonary sarcoidosis from tuberculosis can sometimes be very difficult, since they have pathological similarities. The finding of necrotizing granuloma cannot exclude pulmonary sarcoidosis, because it was reported that 6% of sarcoidosis patients had necrotizing granulomas [2]. There are recent reports suggesting that *Propionibacterium acnes* (*P. acnes*) might have an etiologic association with sarcoidosis [3-7]. The investigators used a *P. acnes*-specific monoclonal antibody (PAB antibody) that detected small round bodies in sarcoid granulomas with high frequency and specificity [3,4]. The PAB antibody did not react with tuberculous granulomas.

We herein present a patient with pulmonary sarcoidosis with necrotizing granulomas, whose findings on initial laboratory testing were not typical of sarcoidosis, but for whom immunohistochemical staining using an antibody to *P. acnes* was useful to perform correct diagnosis.

Case Report

A 45-year-old Japanese woman who was a former smoker presented to our hospital with a 1 month history of cough. On her first visit to our clinic, she was afebrile, and chest auscultation was negative for wheezing and rales. Chest computed tomography (CT) revealed an irregular nodular shadow in the right upper pulmonary lobe, which suggested either pulmonary sarcoidosis or tuberculosis

(Figure 1A). Gallium-67 scintigraphy was negative for gallium uptake. The laboratory findings are presented in Table 1. In brief, no obvious abnormalities were observed on initial laboratory testing, including serum levels of calcium and angiotensin-converting enzyme (ACE), or the presence of antineutrophil cytoplasmic antibody. An interferon-gamma release assay (QuantiFERON[®]-TB Gold) was also negative. A transbronchial lung biopsy (TBLB) of a nodule showed eosinophilic necrotizing granulomas that stained negative for acid-fast bacilli (Figure 1B). Analysis of bronchoalveolar lavage fluid (BALF) showed a total cell count of 2.78×10^5 cells/mL, with 11.4% lymphocytes and a CD4/CD8 ratio of 1.98. The cultures of TBLB and BALF were negative for bacterial growth, including acid-fast bacilli, and fungal growth. These results were inconclusive for differentiating between pulmonary sarcoidosis and tuberculosis.

However, immunostaining of a biopsy specimen using a *P. acnes*-specific monoclonal antibody (PAB antibody) revealed many small round bodies in the granulomas (Figure 1C). Based on this result, we diagnosed the patient clinically with sarcoidosis. The patient was prescribed ciclesonide inhalation (400 µg twice daily) and followed closely. Seven months after she started treatment, her cough resolved, and her chest radiograph showed marked improvement (Figure 1D), which was not compatible with the time course of active tuberculosis.

Discussion

There are a variety of pulmonary granulomatous diseases,

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Received February 16, 2016; **Accepted** April 14, 2016; **Published** April 18, 2016

Citation: Suzuki T, Fujita A, Takamori M, Murata K, Wada A, et al. (2016) Implication of Immunohistochemistry for *Propionibacterium acnes* in Differential Diagnosis of Necrotizing Granuloma. J Pulm Respir Med 6: 335. doi:[10.4172/2161-105X.1000335](https://doi.org/10.4172/2161-105X.1000335)

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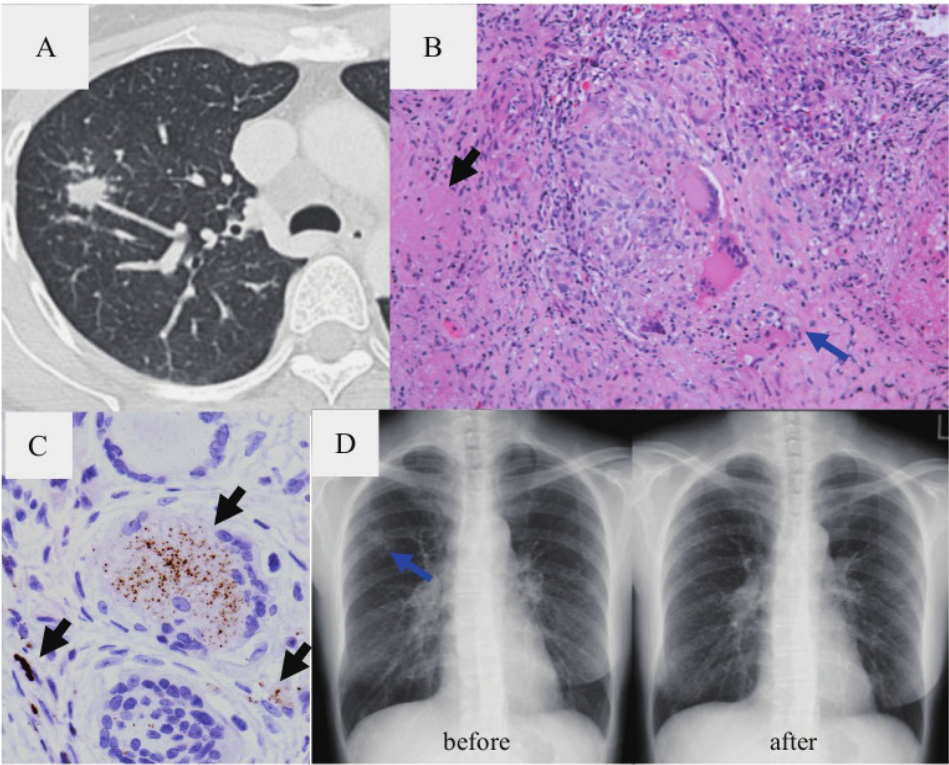


Figure 1: **A:** Chest computed tomography on the patient's first visit to our clinic revealed an irregular nodular shadow in the right upper pulmonary lobe. **B:** A transbronchial lung biopsy (TBLB) specimen from the nodule showed necrotizing granulomas (hematoxylin-eosin stain; original magnification, X400). Necrosis is indicated by the black arrow, and the granuloma with Langhans giant cells is indicated by the blue arrow. **C:** *Propionibacterium acnes* organisms within a sarcoid granuloma of the transbronchial lung biopsy (TBLB) specimen. Immunostaining with *Propionibacterium acnes*-specific monoclonal antibody (PAB antibody) is shown (original magnification, X1000). Many small round bodies are seen within the granulomas (black arrows). **D:** Chest radiographs before and after treatment with inhaled ciclesonide. The abnormal shadow in the right upper lung field (blue arrow) disappeared after the treatment.

Hematology		Biochemistry		Serology	
WBC	5;100/mL	TP	8.0 g/dL	CRP	0.74 mg/dL
Neutrophil	59.0%	Alb	4.4 g/dL	ACE	14.9 U/mL
Lymphocyte	28.0%	AST	21 IU/L	MPO-ANCA	<10 EU
Monocyte	8.0%	ALT	19 IU/L	PR3-ANCA	<10 EU
Eosinophil	5.0%	LDH	208 IU/L	β-D glucan	<3.14 pg/mL
Basophil	0.0%	ALP	258 IU/L		
RBC	419 × 10 ⁴ /mL	BUN	11.2 mg/dL		
Hb	12.8 g/dL	Cre	0.55 mg/dL		
Hct	38.5 %	Na	141 mEq/L		
Plt	207;000/mL	K	3.8 mEq/L		
		Cl	110 mEq/L		
		Ca	9.5 mg/dL		

Table 1: Laboratory data on admission (ACE: Angiotensin-Converting Enzyme; Alb: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BUN: Blood Urea Nitrogen; Ca: Calcium; Cl: Chlorine; Cre: Creatinine; CRP: C-Reactive Protein; Hb: Hemoglobin; Hct: Hematocrit; K: Kalium; LDH: Lactate Dehydrogenase; MPO-ANCA: Myeloperoxidase Antineutrophil Cytoplasmic Antibody; Na: Natrium; Plt: Platelet; PR3-ANCA: Proteinase-3 Antineutrophil Cytoplasmic Antibody; RBC: Red Blood Cell; TP: Total Protein; WBC: White Blood Cell).

including sarcoidosis, tuberculosis, fungal disease, granulomatosis with polyangiitis, collagen disease, autoimmune disease, and hypersensitivity pneumonitis. Among these conditions, differentiating between pulmonary sarcoidosis and tuberculosis is very difficult, since they have clinical and radiological similarities. The pathological hallmark of sarcoidosis is noncaseating granuloma, but some patients have been reported with necrotizing granuloma [2].

The etiology of sarcoidosis is still unknown. However, it has been reported to be a result of an abnormal immune response to certain

antigenic stimuli. *Mycobacterium tuberculosis* and *P. acnes* are the most commonly implicated causative organisms [8]. To date, *P. acnes* is the only microorganism that has been isolated from bacterial cultures of specimens from sarcoid lesions [8,9]. Negi et al. recently reported that *P. acnes* were detected with high frequency and specificity in sarcoid granulomas by a *P. acnes*-specific monoclonal antibody (PAB antibody) that reacted with cell-membrane-bound lipoteichoic acid [3]. Sarcoidosis may arise from a Th1 immune response to *P. acnes* in association with a hereditary or acquired abnormality of the immune

system [9].

Sarcoidosis sometimes represents a diagnostic challenge. The work up of our patient revealed the following atypical findings: 1) necrotizing pulmonary granuloma, 2) normal ACE level, 3) negative gallium uptake, and 4) relatively low proportion of lymphocytes and CD4/CD8 ratio in BALF. There were also no extrapulmonary manifestations of sarcoidosis without mediastinal lymphadenopathy. Although the interferon-gamma release assay was negative, a negative result by itself could not rule out active tuberculosis. The interferon-gamma release assay is not the gold standard for the diagnosis of tuberculosis, and a positive result only supports the diagnosis. Moreover, the CT findings were compatible with tuberculosis, showing endobronchial spread along adjacent airways and relatively well defined nodules with a tree-in-bud pattern. In such cases, an antibody to *P. acnes* such as the PAB antibody can be useful for supporting the diagnosis of sarcoidosis. Investigators using the PAB antibody found that it did not react with nonsarcoid granulomas in any of 45 tuberculosis samples or 34 samples from sarcoid reactions [3]. Our patient's cough resolved and her chest radiograph showed improvement after treatment using inhaled ciclesonide for 7 months. Although we were unable to determine if the inhaled ciclesonide led to her improvement, the course of her illness was not compatible with active tuberculosis.

The present report also needs to keep necrotizing sarcoid granulomatosis (NSG) on the list of differential diagnoses to be considered for pulmonary sarcoidosis. NSG is an extremely rare granulomatosis, only about 200 cases were reported in English literature in pubmed [10]. Its pathogenesis still remains unclear. Diagnosis of NSG mainly relies on pathological findings. Non-caseating epithelial cell granulomas of NSG may invade the vessel wall and lumen to form granulomatous vasculitis and lesions of coagulation necrosis; lymphocytes, multinucleated giant cells and other inflammatory cells can infiltrate pulmonary blood vessel walls and cause severe stenosis or occlusion of the vessel lumen with perivascular granuloma formation, all of which were not evident in this case [10]. However, some argued that NSG might be a subtype of sarcoidosis [11,12]. Indeed, there was a report of NSG with *P. acnes* DNA found in the granulomas of lung specimens [13].

In summary, we reported a case of pulmonary sarcoidosis that was difficult to differentiate from tuberculosis and were successfully diagnosed by *P. acnes*-specific immunostaining. The presence of

necrosis in a granuloma does not exclude the diagnosis of sarcoidosis. Evaluation by *P. acnes*-specific immunostaining may be useful for differentiating between granulomatous diseases.

Acknowledgements

We are grateful to Tamiko Takemura (Department of Pathology, Japanese Red Cross Medical Center, Japan) for her contributions to pathological assessment.

References

1. <http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou03/13.html>
2. Danila E, Zuraszka E (2008) Diagnostic value of epithelioid cell granulomas in bronchoscopic biopsies. Intern Med 47: 2121-2126.
3. Negi M, Takemura T, Guzman J, Uchida K, Furukawa A, et al. (2012) Localization of propionibacterium acnes in granulomas supports a possible etiologic link between sarcoidosis and the bacterium. Mod Pathol 25: 1284-1297.
4. Eishi Y (2013) Etiologic link between sarcoidosis and Propionibacterium acnes. Respir Investig 51: 56-68.
5. Baughman RP, Lower EE, du Bois RM (2003) Sarcoidosis. Lancet 361: 1111-1118.
6. de Brouwer B, Velkamp M, Wauters CA, Grutters JC, Janssen R (2015) Propionibacterium acnes isolated from lymph nodes of patients with sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 32: 271-274.
7. Zhou Y, Hu Y, Li H (2013) Role of Propionibacterium Acnes in Sarcoidosis: A Meta-analysis. Sarcoidosis Vasc Diffuse Lung Dis 30: 262-267.
8. Homma JY, Abe C, Chosa H, Ueda K, Saegusa J, et al. (1978) Bacteriological investigation on biopsy specimens from patients with sarcoidosis. Jpn J Exp Med 48: 251-255.
9. Abe C, Iwai K, Mikami R, Hosoda Y (1984) Frequent isolation of Propionibacterium acnes from sarcoidosis lymph nodes. Zentralbl Bakteriol Mikrobiol Hyg A 256: 541-547.
10. Huang H, Li C, Bai C, Li Q, Zheng W, et al. (2013) Necrotizing sarcoid granulomatosis with hemoptysis: a case report and literature review. Diagn Pathol 8: 79.
11. Schumacher C, Lohr A, Kaup M, Walter P, Roth J, et al. (2011) Necrotizing sarcoid granulomatosis: ocular manifestation in 3 children of African origin. Arch Ophthalmol 129: 1370-1372.
12. Strickland-Marmol LB, Fessler RG, Rojiani AM (2000) Necrotizing sarcoid granulomatosis mimicking an intracranial neoplasm: clinicopathologic features and review of the literature. Mod Pathol 13: 909-913.
13. Arai T, Inoue Y, Eishi Y, Yamamoto S, Sakatani M (2008) Propionibacterium acnes in granulomas of a patient with necrotizing sarcoid granulomatosis. Thorax 63: 90-91.