Successful Additional Corticosteroid Treatment in a Patient with *Mycoplasma pneumoniae* Pneumonia in whom a Monobacterial Infection was Confirmed by a Molecular Method Using Bronchoalveolar Lavage Fluid

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Abstract

A 23-year-old Japanese woman was admitted to A hospital due to pneumonia. IgM for *Mycoplasma pneumoniae* was positive, and the patient was treated with imipenem/cilastatin, clindamycin, pazufloxacin and minocycline. However, both the chest radiological findings and the symptoms became exacerbated, and she was therefore transferred to our hospital. The bronchoalveolar lavage fluid was obtained, and a 16S rRNA gene sequencing analysis revealed a monobacterial infection of *Mycoplasma pneumoniae*. Therefore, corticosteroid treatment in addition to minocycline was administered, and the patients symptoms, laboratory data and chest radiographs improved. Corticosteroid therapy may therefore be considered for patients with refractory *M. pneumoniae* pneumonia.

Key words: *Mycoplasma pneumoniae*, 16S ribosomal RNA gene, clone library sequencing analysis, corticosteroid treatment

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Introduction

Mycoplasma pneumoniae pneumonia is one of the common types of atypical pneumonia and is typically successfully treated with macrolides, respiratory quinolone, and tetracycline; however, it may occasionally worsen even under proper antimicrobial treatment. *M. pneumoniae* pneumonia is essentially a reaction of the host defense as well as infectious aspects, however, excessive reactions of the host immunity may exacerbate the pneumonia, and the precise mechanism of exacerbation of pneumonia in severe *M. pneumoniae* pneumonia has not yet been fully elucidated. We herein present a patient with *M. pneumoniae* pneumonia who was confirmed to have a monobacterial infection of *M. pneumoniae* by a 16S ribosomal RNA gene sequencing

analysis and bacterial cell count using ethidium bromide staining of the bronchoalveolar lavage fluid. Although the initial treatment with appropriate antimicrobials for *M. pneumoniae* pneumonia was unsuccessful, the patient was successfully treated with additional systemic corticosteroid treatment.

Case Report

A 23-year-old Japanese woman visited A hospital due to a fever, general fatigue and a productive cough in the middle of May. She had no history of smoking, drinking or any particular medical history and had no comorbid diseases, including allergic diseases or family medical histories. Chest computed tomography (CT) performed on admission revealed new areas of infiltrations and a tree-in-bud appear-

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Table. The Laboratory Findings on Admission.

< Blood cell counts >		< Blood chemistry >		< Serology >	
WBC	7,200 /uL	TP	5.4 g/dL	CRP	20.1 mg/dL
Neu	80.1 %	Alb	2.1 g/dL	Cold agglutinin	×8,192
Lym	9.5 %	T-Bil	0.5 mg/dL	<i>Mycoplasma pneumoniae</i>	
Mono	4.0 %	AST	80 IU/L	(CF)	×512
Eos	31.0 %	ALT	47 IU/L	<i>Chlamydia pneumoniae</i> (IgG)	(-)
Baso	0.5 %	LDH	657 IU/L	<i>Chlamydia pneumoniae</i> (IgA)	(-)
RBC	397 ×10 ⁶ /uL	γ-GTP	32 IU/L	ImmunoCard	
Hb	10.4 g/dL	T-cho	133 mg/dL	<i>Mycoplasma</i> test	(+)
Ht	31.6 %	TG	91 mg/dL		
Plt	19.3 ×10 ⁴ /uL	BUN	4 mg/dL		
		Cr	0.3 mg/dL	< Urinalysis >	
< Blood gas analysis (nasal O ₂ 2L) >		CK	514 IU/L	<i>Legionella pneumophila</i> Ag	(-)
pH	7.523	Na	132 mEq/L	<i>Streptococcus pneumoniae</i> Ag	(-)
PaO ₂	75.0 mmHg	K	4.5 mEq/L		
PaCO ₂	31.8 mmHg	ESR	88 mm/h	< Bronchoalveolar lavage >	
HCO ₃ ⁻	25.6 mEq/L			Gram staining	no organisms
BE	3.4			Culture	no organisms

ance in the right upper lobe with right pleural effusion. Upon admission to A hospital, her transcutaneous saturation of oxygen was 97% (room air) and her body temperature was 40.3°C. The laboratory findings demonstrated a white blood cell count within the normal range (5,800/ μ L), an increased serum C-reactive protein (CRP) level (23.4 mg/dL) and a positive result for the *Mycoplasma pneumoniae* IgM antibody test. According to these findings, the patient was diagnosed with *M. pneumoniae* pneumonia. Treatment with pazufloxacin (PZFX, 1 g/day) was initiated on the day of admission, however, her chest X-ray and fever pattern worsened. PZFX was changed to imipenem/cilastatin and clindamycin (IPM/CS, 1 g/day and CLDM, 1 g/day) on the 4th day of admission, followed by an additional treatment of minocycline (MINO, 200 mg/day) on the 6th day of admission. However, the infiltrations in the right lung, hypoxemia, an increased fever and dyspnea all continued to worsen.

The patient was then transferred from A hospital to our hospital for further treatment. No abnormal findings were audible in the bilateral lung fields on auscultation. The laboratory findings on this admission (Table) demonstrated a normal white blood cell count (7,200/ μ L), increased serum levels of lactate dehydrogenase (LDH) (657 IU/mL) and CRP (20.1 mg/dL), hypoalbuminemia (2.1 g/dL) and hypoxemia (partial pressure of arterial oxygen (PaO₂) 75 mmHg on nasal oxygen support of 2 L/min). The chest X-ray (Fig. 1A) and CT (Fig. 1B, C) on the day of transfer to our hospital revealed consolidations in the right upper and middle lung fields with bilateral pleural effusions and reticulonodular shadows in the left lingular segment. The treatment with MINO (200 mg/day) was continued, and a bronchoalveolar lavage fluid (BALF) specimen was obtained from the right S₃ using fiberoptic bronchoscopy on the same day. The bacterial composition of the BALF specimen was analyzed using a clone library analysis of the 16S rRNA gene and the total bacterial cell counts were determined using epifluorescence microscopy as previously described (1).

Briefly, DNA samples were extracted from the BALF specimen and 580 partial base pairs of the 16S rRNA gene were amplified via polymerase chain reaction (PCR) using specific primer pairs. The PCR products were then cloned with a TOPO TA cloning kit (Invitrogen; Carlsbad, USA), and the clone library was constructed. Ninety-six clones were randomly chosen from the clone library, and the sequences of the 16S rRNA gene were determined. A homology search comparing the sequences with the recorded reference strains was performed using the basic local alignment search tool (BLAST) algorithm. In addition, ethidium bromide staining was used to determine the bacterial cell count in the sample. As a result, 98.6% (68/69 clones) of the bacterial clones in the sample determined using this molecular method were identified to be *M. pneumoniae*, and the numbers of bacteria using ethidium bromide staining was 4.6×10⁶ cells/mL, however, the conventional cultivation methods of the BALF did not identify any pathogens. According to these results, the patient was confirmed to have monobacterial pneumonia caused by *M. pneumoniae* four days after the day of transfer. She still had a high-grade fever and high serum CRP levels with unchanged chest X-ray findings six days after this admission. Therefore, additional treatment with prednisolone (PSL, 40 mg/day) was started in combination with MINO (200 mg/day). Following this combination therapy, the patient's symptoms, such as the fever and cough, laboratory data (CRP levels) and the chest radiologic findings gradually improved (Fig. 2). PSL was tapered, MINO and PSL were eventually discontinued 12 days after the transfer day, and the patient was discharged without any complications.

Discussion

In the present patient, additional treatment with corticosteroid dramatically improved the *M. pneumoniae* pneumonia even under appropriate antimicrobial treatment. In ad-

Figure 1. A chest X-ray (A) and computed tomography findings (B and C) obtained on the day of transfer from A hospital to our hospital show the areas of abnormal opacities in the right middle lung field and consolidations and centrilobular nodular shadows in the right upper and middle lobe and bilateral pleural effusions, respectively.

Figure 2. The clinical course of the patient. MINO: minocycline, PSL: prednisolone.

dition, the 16S rRNA gene sequencing analysis and ethidium bromide staining of the BALF specimen obtained from the right S³ identified the monobacterial infection of *M. pneumoniae* in the present patient.

M. pneumoniae infection is common in community-acquired pneumonia among young adults and children (1). Most patients infected with *M. pneumoniae* do not develop severe pneumonia. In fact, only 3 to 10% of the infected patients actually develop pneumonia (2).

M. pneumoniae infection is established by the attachment of the bacteria to the surface membrane of ciliated epithelial

cells, and the direct injury of the alveolar epithelial cells and pulmonary interstitium (3). *M. pneumoniae* lack a cell wall and are covered by many lipoproteins. The pathogenesis of *M. pneumoniae* is believed to be immune-mediated induced by these lipoproteins rather than induced directly by the bacteria. The lipoproteins of *M. pneumoniae* are recognized by toll-like receptors (TLR) (e.g., TLR-2/TLR-6 and TLR-1/TLR-2) on alveolar macrophages (4), and these macrophages can be activated and in turn secrete proinflammatory cytokines, such as interleukin (IL)-12, and other chemokines.

M. pneumoniae pneumonia may occasionally develop into severe pneumonia, progress to respiratory failure and even occasionally be fatal, despite the administration of appropriate antibiotic treatment, even in healthy subjects. Previous reports demonstrated that only 2% of all patients with *M. pneumoniae* pneumonia required hospitalization (5), and approximately 0.5-10.9% of the inpatients required mechanical ventilation (6, 7). The comprehensive mechanism of the severe respiratory distress in patients with *M. pneumoniae* pneumonia had been unclear thus far, however, several reports have estimated that it may be due to mononuclear cell migration into the airway, which results in pulmonary infiltrates. *M. pneumoniae* can be phagocytized by the macrophages after opsonization of *M. pneumoniae* by complement or antibodies. The macrophages are activated to release cytokines and chemokines, and mononuclear cell infiltrations (via T- and B-cells or plasmacytes) to the lung occurs (5, 8-12). The immune response is then amplified by the further release of proinflammatory chemical mediators (9). Tanaka et al. (13) demonstrated that the serum levels of IL-18, which promotes the induction of Th1 cytokines, thus leading to the activation of alveolar macrophages and the cellular immune response, were elevated during the acute phase of *M. pneumoniae* pneumonia. Moreover, circulating IL-18 values had a positive correlation with the number of affected pulmonary lobes.

The downregulation of the cell-mediated immune response by corticosteroids has been previously reported (14, 15) and therefore the additional corticosteroid treatment during severe *M. pneumoniae* pneumonia may have a favorable effect by reducing the hyperimmune-mediated pulmonary injury. In addition, corticosteroids downregulate the immune responses mediated by Th1 cells, and therefore corticosteroids may reduce the immune-mediated pulmonary injury induced by *M. pneumoniae*. Animal experimental models have shown that corticosteroids downregulate the host immune response. Furthermore, treatment with the combination of clarithromycin and a corticosteroid, compared to clarithromycin alone, resulted in a significant reduction of IL-12, p40 and regulated on activation, normal T cell expressed and secreted (RANTES) (16). Izumikawa reported that the respiratory functions of a majority of fulminant *M. pneumoniae* pneumonia patients were successfully improved with corticosteroid treatment within 3-5 days, which was also considered to be due to a suppressive effect of the corticosteroids on hyper-activated cellular immunity (17). In children, You et al. (18) demonstrated that three-day methylprednisolone pulse therapy improved the clinical symptoms, radiological findings and CRP levels in severe refractory childhood *M. pneumoniae* pneumonia. In adults, Miyashita et al. (19) demonstrated that in 227 adults with *M. pneumoniae* pneumonia, 13 patients required intensive care unit admission due to acute respiratory failure. In addition, all the cases received corticosteroids with adequate antibiotics, and their conditions promptly improved.

The molecular method we used (1, 20) can be used to

identify pathogens that are generally difficult to cultivate using the ordinary cultivation methods, such as *M. pneumoniae*, and was identical for evaluating the bacterial flora in the sample. In general, complicated or refractory *M. pneumoniae* pneumonia tends to be considered to be a mixed infection of *M. pneumoniae* and other bacteria, therefore broad-spectrum antibiotics in addition to the agents effective for treating *M. pneumoniae* tend to be administered. In particular, a mixed bacterial infection with *M. pneumoniae* has been considered in all patients who show severe respiratory failure due to *M. pneumoniae* pneumonia. However, thus far, no previous reports of *M. pneumoniae* pneumonia that was successfully treated with corticosteroid and strictly confirmed a monobacterial infection caused by *M. pneumoniae* have been reported. In the present patient, the molecular method using the BALF specimen revealed that the pneumonia of the present patient was due to a monobacterial infection of *M. pneumoniae*. In addition to the molecular analysis using the 16S rRNA gene, we used ethidium bromide staining to perform bacterial cell counting in the sample (1), and the total cell count of the specimen obtained from the BALF sample was 4.6×10^6 cells/mL. This number of bacterial cells was more than the median (2.6×10^6 cells/mL) of bacterial cell numbers in the BALF samples in patients with community-acquired pneumonia before starting antimicrobial treatment (1). Even after receiving antimicrobial treatment with PZFX and MINO for approximately one week, a considerable number of *M. pneumoniae* was still observed in the BALF, and additive systemic corticosteroid treatment with MINO showed a prompt favorable improvement. According to the results of the BALF sample and the clinical response to additive systemic corticosteroid treatment in this patient, it is speculated that an excessive biological response to a relatively large amount of bacterial cells that might be viable or inviable may exacerbate the disease condition in refractory patients with *M. pneumoniae* pneumonia, and additive systemic corticosteroid treatment may suppress this excessive biological reaction, thus leading to a favorable clinical outcome. Therefore, additive systemic corticosteroid treatment may occasionally be effective in some patients with severe or refractory *M. pneumoniae* pneumonia.

In conclusion, we herein presented a case of successful additional corticosteroid treatment of a monobacterial infection of *M. pneumoniae* pneumonia, which was confirmed by the clone library analysis of the 16S rRNA gene and ethidium bromide staining using the BALF specimen. Additive systemic corticosteroid treatment to proper antimicrobials should occasionally be considered for the treatment of patients with severe or refractory *M. pneumoniae* pneumonia.

The authors state that they have no Conflict of Interest (COI).

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