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Abstract

Pathologic features of Mycoplasma pneumoniae infection (M. pneumonia) are generally nonspecific, and the literature regarding the pathologic features of M. pneumonia with intraalveolar exudates is limited. Clinical and histopathological studies were performed in 3 patients with M. pneumonia which did not respond to erythromycin and minocycline, but all rapidly recovered after corticosteroid therapy. In pathologic findings, we observed intraalveolar exudates and focal organization in M. pneumonia, and its intraalveolar lesions were compared between M. pneumonia and bronchiolitis obliterans organizing pneumonia containing fibrin (BOOP). Immunohistochemical studies were performed using the streptavidin biotin peroxidase complex method with anti-alpha-smooth muscle actin antibody and anti-pancytokeratin AE1/AE3 antibody. In pathologic findings, more fibrin deposits in intaalveolar lesions were observed in M. pneumonia than in BOOP. In intaalveolar lesions of M. pneumonia, a larger amount of nuclear debris, more neutrophils, and more erythrocytes were noted. Myofibroblasts were observed in the organization of BOOP, while in the intaalveolar lesions of M. pneumonia, myofibroblasts were not observed. These results suggest that M. pneumonia with intraalveolar exudates responds well to corticosteroid and its intraalveolar lesions apparently differed from those in BOOP.

KEYWORDS: exudate, fibrin, Mycoplasma pneumonia, organizing pneumonia, steroid therapy

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Case Report

A Study on Intraalveolar Exudates in Acute Mycoplasma Pneumoniae Infection

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Pathologic features of Mycoplasma pneumoniae infection (M. pneumonia) are generally non-specific, and the literature regarding the pathologic features of M. pneumonia with intraalveolar exudates is limited. Clinical and histopathological studies were performed in 3 patients with M. pneumonia which did not respond to erythromycin and minocycline, but all rapidly recovered after corticosteroid therapy. In pathologic findings, we observed intraalveolar exudates and focal organization in M. pneumonia, and its intraalveolar lesions were compared between M. pneumonia and bronchiolitis obliterans organizing pneumonia containing fibrin (BOOP). Immunohistochemical studies were performed using the streptavidin biotin peroxidase complex method with anti-α-smooth muscle actin antibody and anti-pancytokeratin AE1/AE3 antibody. In pathologic findings, more fibrin deposits in intaalveolar lesions were observed in M. pneumonia than in BOOP. In intaalveolar lesions of M. pneumonia, a larger amount of nuclear debris, more neutrophils, and more erythrocytes were noted. Myofibroblasts were observed in the organization of BOOP, while in the intaalveolar lesions of M. pneumonia, myofibroblasts were not observed. These results suggest that M. pneumonia with intraalveolar exudates responds well to corticosteroid and its intraalveolar lesions apparently differed from those in BOOP.

Key words: exudate, fibrin, Mycoplasma pneumonia, organizing pneumonia, steroid therapy

Mycoplasma pneumoniae infection (M. pneumonia) is a mild disease with a favorable prognosis as a rule, but recently, severe cases showing acute respiratory insufficiency have been reported [1, 2]. Reported data suggest that the pathologic features of M. pneumonia are not specific in general, and are frequently similar to those of bronchiolitis or interstitial pneumonia [3-6]. The literature on the pathologic features of M pneumonia is limited. Rollins S. reported that M. pneumonia showed a polymorpho-nuclear leukocyte-rich exudate in the bronchiolar lumina and a lymphoplasmacytic bronchiolar wall infiltrate [7]. We report here the clinical features and histopathological findings of 3 patients with M. pneumonia which did not respond to erythromycin and minocycline, but all rapidly recovered after corticosteroid therapy. Additionally, the pathological findings of intraalveolar lesions were compared between M. pneumonia and idiopathic bronchiolitis obliterans organizing pneumonia associating fibrin exudation (BOOP) because we observed characteristic intraalveolar exudates and only

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focal organization in M. pneumonia compared to those of BOOP.

Methods

Lung specimens obtained by transbronchial lung biopsy (TBLB) or open lung biopsy were fixed in 10% buffered formalin, then stained with H-E and phosphotungstic acid-hematoxylin (PTAH) for fibrin. Immunohistochemical studies of dewaxed lung sections were performed using the streptavidin biotin peroxidase complex method (ABC method) according to a kit manual employing anti-α-smooth muscle actin (α-SMA) antibody to detect myofibroblast in intraalveolar spaces (DAKO, CA, USA, 1:200) and anti-pancytokeratin AE1/AE3 antibody to detect alveolar epithelial cell proliferation (Boehringer-Mannheim, Biochemica, Mannheim, Germany, 1:400).

Results

Case Report. Case 1: A 44-year-old male was admitted to the hospital because of 8 days history of high fever and cough. Physical examination revealed tachycardia and high fever (38.4 ˚C). Laboratory findings on admission were as follows: Hb 13.2 g/dl; white blood cell (WBC) count, 10,300/μl (neutrophils 61%, lymphocytes 22%, eosinophils 2%, monocytes 12%); erythrocyte sedimentation rate (ESR), 77 mm/h; C-reactive protein (CRP), 10.1 mg/dl. Chest X-ray revealed infiltrative shadows partly including particulate shadows in the right upper lung field and left lower lung field (Fig. 1A). Sputum examination showed normal findings. Cold agglutinin titer was 1:128, and Mycoplasma antibody titer (CF) was 1:256 (at admission) and 1:1.024 (20 days later). Although erythromycin was administered for 12 days, symptoms persisted, and there was no change in infiltrative shadows on chest X-ray films. TBLB was performed for accurate diagnosis. Pathologic findings of biopsied specimens taken from the left S10 revealed a number of intraalveolar exudates with marked fibrin deposition and focal organization, in which fibroblastic spindle cell proliferation was observed, containing many erythrocytes, neutrophils, and much nuclear debris. In the surrounding lung field, marked hyperplasia of the alveolar epithelium was noted. Therapy with oral predonisone (PSL 20 mg) was administered. As a result, subjective symptoms dramatically improved, and shadows on chest X-ray films and computed tomography of the lungs (CT) disappeared (Fig. 1B).

Case 2: A 37-year-old male was admitted to the hospital because of 7 days history of high fever, cough, and dyspnea. Physical examination demonstrated tachycardia, hyperpnea, high fever (38.7 ˚C), and fine crackle. Laboratory findings on admission were as follows: Hb 13.9 g/dl; WBC 7,800/μl (neutrophils 88%, lymphocytes 8%, eosinophils 2%, monocytes 2%); ESR 80 mm/h; CRP 6 mg/dl; PO_2 54.5 Torr; and PCO_2 37.4 Torr. Chest X-ray revealed infiltrative

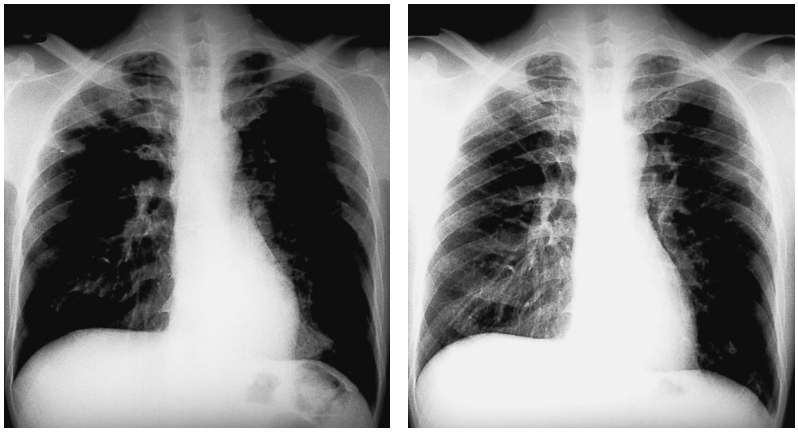

Fig. 1 A, a chest roentgenogram obtained on admission reveals infiltrative shadows partly including particulate shadows in the right upper and left lower lung fields of case 1. B, a chest roentgenogram after corticosteroid therapy revealed a marked improvement in case 1.

shadows partly including particulate shadows in the bilateral lower lung fields. All results of a tuberculin test and sputum examination were negative. Mycoplasma antibody titer (CF) was 1:128 (at admission) and 1:2,048 (14 days later). The patient was treated with erythromycin and flomoxef sodium for 10 days, but high fever and dyspnea persisted and infiltrative shadows on chest X-ray films were aggravated. Open lung biopsy was carried out for precise diagnosis. Pathologic findings of tissue specimens taken from right S10 revealed a number of intraalveolar exudates containing fibrin deposition with focal organization. Corticosteroid pulse therapy was administered, resulting in dramatic improvement of the subjective symptoms, and disappearance of shadows on chest X-rays and CT.

Case 3: A 28-year-old female was admitted to the hospital because of 5 days history of high fever and cough. Physical examination showed high fever (38.5 ℃). Laboratory findings on admission were as follows: Hb 13 g/dl; WBC 8,800/μl (neutrophils 81%, lymphocytes 15%, eosinophils 1%, monocytes 3%); ESR 68 mm/h; CRP 6.8 mg/dl. Chest X-ray revealed infiltrative shadows in the left middle lung field. Mycoplasma antibody titer (CF) was 1:128 (at admission), and 1:512 (10 days later). The patient received erythromycin for 11 days, but high fever persisted, and infiltrative shadows on chest X-rays remained unchanged. TBLB was carried out for accurate diagnosis. Pathologic findings of tissue specimens taken from the left S4 revealed the same as in both cases 1 and 2. Therapy with oral predonisone (PSL 20 mg) was administered, resulting in dramatic improvement of the subjective symptoms, and disappearance of shadows on chest X-rays and CT.

Pathological findings (Table 1). Pathologically, all 3 patients exhibited almost the same findings, revealing common characteristics of intraalveolar exudates with focal organization. Hence, the main pathological findings of intraalveolar lesions were compared between M. pneumonia and BOOP. H-E staining showed more fibrin deposits in intraalveolar exudates of M. pneumonia than in intraalveolar lesions of BOOP. Furthermore, in intraalveolar exudates of M. pneumonia, more erythrocytes, neutrophils, and a larger amount of nuclear debris were also noted (Fig. 2A). In contrast, intraalveolar lesions of BOOP included a few macrophages, lymphocytes, and neutrophils (Fig. 3A). Fig. 2B and 3B illustrate PTAH staining patterns, showing more fibrin deposits in intraalveolar exudates of M. pneumonia than in intraalveolar lesions of BOOP (Figs. 2B, 3B). As shown in Fig. 3C, with regard to fibroblasts in the intraalveolar lesions, staining with α-SMA antibody demonstrated proliferation of myofibroblasts positive for α-SMA antibody in intraalveolar organization of BOOP (Fig. 3C), while in intraalveolar lesions of M. pneumonia, myofibroblasts were not observed (Fig. 2C). Staining with anti-pancytokeratin AE1/AE3 antibody revealed that the surface of the intraalveolar organization was covered with type II alveolar epithelium in patients with BOOP, while in patients with M. pneumonia, such covering was not noted at all (Figs. 2D, 3D). The results described above are summarized in Table 1. Intraalveolar lesions in patients with M. pneumonia obviously differed from those in patients with BOOP.

Table 1 Comparison of pathological findings of both M. pneumonia with intraalveolar exudates and BOOP

	M. pneumonia	*BOOP*
Intraalveolar exudates		
Fibrin	++	+～−
Nuclear debris	+	−
Neutrophil	+	−
Red blood cell	+	+～−
Plasma cell	−	+～−
Macrophage	+	+～−
Myofibroblast	−	+～−
Covering by type II epithelium	−	+
Type II epithelium proliferation in surroundings	++	+

Discussion

M. pneumonia generally responds well to antibiotics and its prognosis is good. So, histologic material is rarely obtained and its classic histologic descriptions are derived from autopsy. The autopsy lung specimen findings in previous reports are septal widening with plasmacytic, lymphocytic infiltrates, necrotizing bronchopneumonias, lymphocytic bronchiolar wall infiltrates, and bronchiolar luminal contents [3–6]. In 1986, Rollins S. studied open lung biopsy specimens from 6 patients with M. pneumonia and reviewed the findings polymorpho-nuclear leukocyte-rich exudates in the bronchiolar lumina, metaplastic cells that lined the bronchioles, a lymphoplasmacytic bronchiolar wall infiltrate, peribronchiolar septal widening, and adjacent hyperplasia of type II pneumocytes [7]. In regard to the intraalveolar lesions

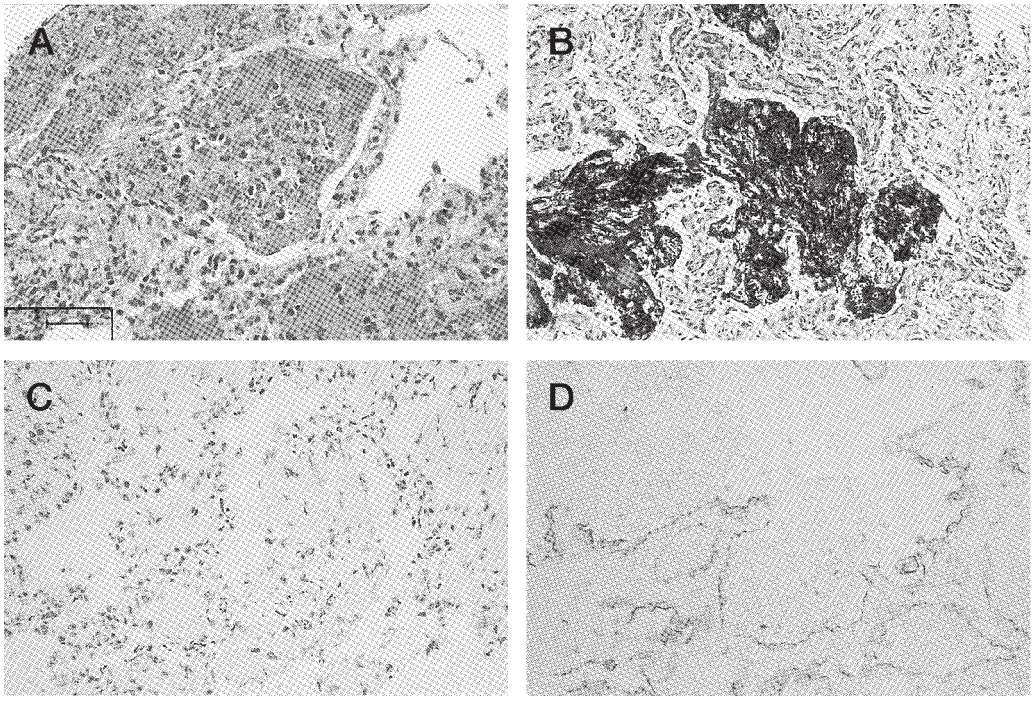

Fig. 2 Intraalveolar lesions in M. pneumonia.
A, intraalveolar exudates contain a large amount of fibrin, neutrophils, red blood cells and nuclear debris, associated with alveolar type II cell proliferation. HE stain × 220.
B, fibrin was positive with phosphotungstic acid hematoxylin (PTAH) stain. PTAH stain × 220.
C, bipolar cells were negative with anti-alpha smooth muscle actin antibody. ABC method × 350.
D, intraalveolar exudates were negative for pancytokeratin antibody, AE1/AE3. ABC method × 350.

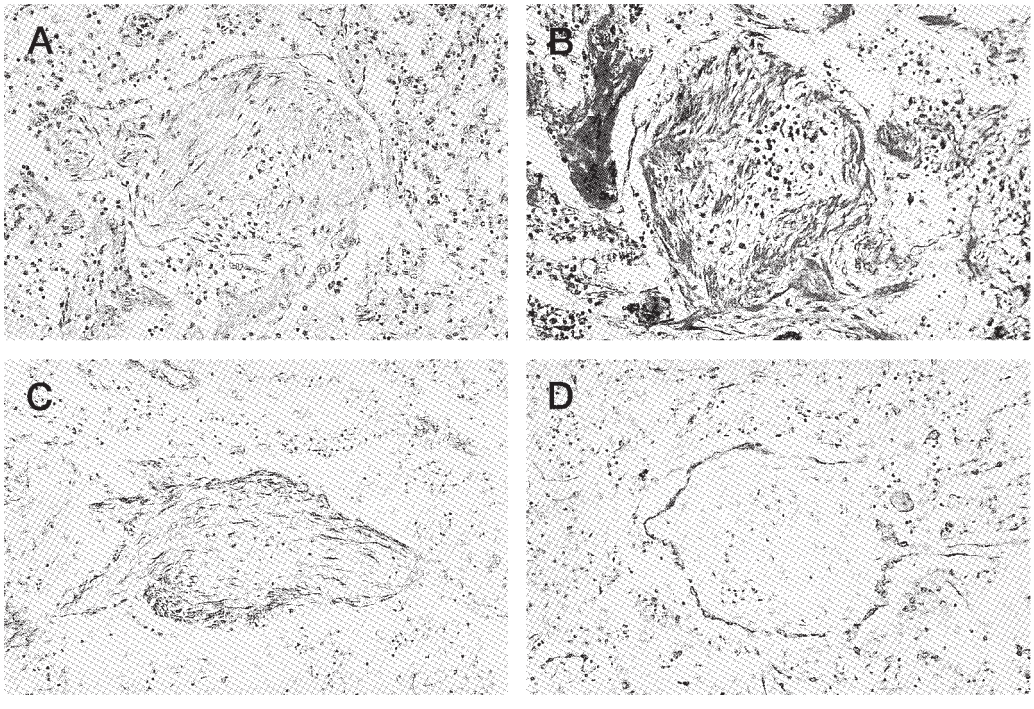

Fig. 3 Intraalveolar lesions in BOOP.
A, intraalveolar lesions mainly consisting of bipolar spindle cells in BOOP. HE stain × 220.
B, spindle cells in intraalveolar lesions were concentrically arranged, revealing PTAH-positivity which suggested myofibroblastic maturity in these spindle cells, but fibrin was not detected. PTAH stain × 220.
C, bipolar cells were positive with anti-alpha smooth muscle actin antibody. ABC method × 350.
D, alveolar type II cells covering intraalveolar organization were positive with antibody to pancytokeratin AE1/AE3. ABC method × 220.

of M. pneumonia, he indicated erythrocytes, macrophages, and neutrophils but not fibrin and nuclear debris. However, detailed pathologic studies of intraalveolar lesions have not been conducted.

In a paper concerning BOOP of M. pneumonia, Epler reported a case of bronchiolitis obliterans (BO) associated with Mycoplasma infection, in which bacterial infection had developed [8], and Coulias described cases of BO associated with the suspicion of M. pneumonia, but the diagnosis was based on high cold agglutinin titers, and there was no description of Mycoplasma antibody titers [9]. Then, cases of BO associated with M. pneumonia were also reported by Prabhu [10]. In addition, Mycoplasma organizing pneumonia (OP) is described by Llibre as bronchiolitis obliterans organizing pneumonia associated with Mycoplasma pneumoniae infection, but detailed pathologic studies have not been reported [11].

We reported 3 patients with M. pneumonia exhibiting intraalveolar exudates with focal organization containing many erythrocytes, neutrophils, and much nuclear debris. Clinical features of M. pneumonia do not differ from those of general Mycoplasma pneumoniae infection, but case 2 was a severe case showing respiratory insufficiency, bearing a closely parallel to the case presented by Llibre in which respiratory insufficiency was present as well. No patients responded to erythromycin or minocycline, but all rapidly recovered after corticosteroid therapy, and M. pneumonia showed characteristics of antibiotic-resistance and steroid-sensitivity.

The pathologic features of M. pneumonia with intraalveolar exudates were common to all 3 patients. Namely, pathologic characteristics of M. pneumonia with intraalveolar exudates included a severe degree of fibrin deposition in intraalveolar exudates, the presence of much nuclear debris, many neutrophils, and many erythrocytes in intraalveolar exudates, the surface of intraalveolar exudates poorly covered with type II alveolar epithelium, and highly developed type II alveolar epithelium in the surrounding lung field. To our knowledge, no studies have investigated pathologic features of M. pneumonia with intraalveolar exudates in detail. As shown in Table 1, these observations seem to indicate that more intense stimulation was indused in the body in patients with M. pneumonia than in patients with BOOP. In patients with BOOP as well, fibrin was sometimes found in intraalveolar lesions, but on such occasions, myofibroblasts grew with absorption of fibrin, promoting organization [12]. However, patients with M. pneumonia with intraalveolar exudates did not demonstrate any proliferation of myofibroblasts except for few fibroblastic spindle cells. In Case 2, the second TBLB performed 10 days after the first lung biopsy revealed the almost complete disappearance of fibrin that had been recognized on the first TBLB, but did not show proliferation of myofibroblasts in intraalveolar lesions. These results suggest that the host defense against Mycoplasma infection may be weaker in patients with M. pneumonia showing intraalveolar exudates than in those with BOOP. In inflammatory processes in patients with M. pneumonia, macrophages are likely to play central roles, because a number of macrophages are generally present in intraalveolar lesions. As the pathogenesis of M. pneumonia, in addition to direct damage by Mycoplasma pneumoniae, the possibility of indirect damage mediated by the immune system is also considered [13, 14]. Therefore, the reason that M. pneumonia with intraalveolar exudates responded well to steroids but poorly to antibiotics may be the weekness of the host defense and the intense stimulation in the delayed immune system as we described. This may also be a reason that M. pneumonia with intraalveolar exudates showed pathologic features different from those of other kinds of M. pneumonia and BOOP. In any event, the distinct differences in pathologic features between M. pneumonia with intraalveolar exudates and BOOP provide interesting insights into the processes of intraalveolar organization. Mycoplasma infection frequently develops in young people. Hence, clinicians must fully evaluate the possibility of M. pneumonia with intraalveolar exudates when patients have complicating Mycoplasma pneumonia. M. pneumonia with intraalveolar exudates differs from M. pneumonia with bronchiolitis and BOOP. Therefore, when antibiotic-resistant M. pneumonia with intraalveolar exudates is suspected, early pathologic diagnosis and corticosteroid therapy are mandatory.

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