Pathological findings of bronchiectases caused by *Mycobacterium avium intracellulare* complex

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**Summary** It has been argued whether bronchiectasis is truly caused by MAC infection or just a predisposed condition in which MAC colonizes. Our present study was designed to evaluate the pathological findings of bronchiectases caused by *Mycobacterium avium intracellulare* complex (MAC) lung infection and to demonstrate MAC in the lesion of bronchiectases. A retrospective study was performed in nine cases with positive cultures for MAC in whom lung resections were performed. A determination of whether or not MAC caused pulmonary disease was made using the 1997 criteria required by the American Thoracic Society. In addition, MAC were cultured from all nine lung specimens. Pathological findings of bronchiectases were evaluated in these nine patients. Destruction of bronchial cartilage and smooth muscles layer, obstruction of airway by granulomas, and ulceration of bronchial mucosa were frequently observed. Our present study demonstrates that destruction of fundamental bronchial structure due to extensive granuloma formation throughout the airways was likely the main cause of bronchiectases in MAC infection.

**Keywords** *Mycobacterium avium intracellulare* complex (MAC) infection; Surgical resection; Pathological findings; Bronchiectases

**Introduction**

The most common established cause of bronchiectasis, defined as irreversible dilatation of the peripheral airways, is infection, often of a chronic or recurrent nature.\(^1\) Necrotizing bacterial infection, mycobacterial infection, and less commonly fungal infections, may result in bronchiectasis.\(^2\) During acute inflammation, the bronchial walls become thickened with inflammatory cells and edema. Ongoing cell-mediated immune response results in an infiltration of lymphocytes, antigen presenting cells, and macrophages.\(^3\) Focal mucosal erosions may develop, resulting in peribronchial abscess formation. Chronic inflammation may
result in destruction of the elastin layer of the bronchial wall, with thinning of the bronchial cartilage, resulting in bronchomalacia.²

It has been argued whether bronchiectasis is truly caused by MAC infection or just a predisposed condition in which MAC colonizes.⁴⁻⁸ Previously, we have reported pathological and radiological findings of operated lungs in patients with MAC infection, and types and distributions of infiltrated cells.⁹ However, in our previous report, description of pathological findings of bronchiectases caused by MAC was very poor. In addition, in previous articles, we have reported that MAC is not demonstrated in the lesion of bronchiectases. Therefore, it is still impossible to conclude that bronchiectases are truly caused by MAC respiratory infection. In addition, it has been demonstrated that MAC can cause hypersensitivity pneumonitis especially in subjects who use a hot tub.¹⁰⁻¹² Therefore, demonstrating MAC in the lesion of bronchiectasis is very important to prove that bronchiectases are truly caused by MAC.

Recently, Fukunaga et al. have demonstrated that detection of mycobacterium using formalin-fixed, paraffin-embedded pathological specimens is often difficult because of reduction of mycobacteria before the staining procedure.¹³ If this evidence is true, although we could not demonstrate MAC using Ziehl–Neelsen staining, MAC may exist as a pathogen. Under this background, the purpose of this study was to evaluate pathological findings of bronchiectases caused by MAC infection and to demonstrate MAC in the lesion of bronchiectases.

Materials and methods

Patients

A retrospective study was performed in nine cases of positive sputum cultured for MAC in whom surgical resection of the lung was performed from February 1989 to August 1999. The criteria for defining NTM pulmonary disease were those of the American Thoracic Society (1997).¹⁴ In addition, MAC were cultured from resected lung specimens in all patients. Characteristics of these nine patients are listed in Table 1. In our report, there were no patients who had AIDS or any underlying lung disease. In four of nine patients, history of dust inhalation was obtained. The ethics committees of the Kagawa Medical University approved the study, and all patients having provided written informed consent before enrollment. The study was performed in accordance with the latest revisions to the Declaration of the Helsinki.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age and sex</th>
<th>Occupation</th>
<th>Date of operation</th>
<th>Operation</th>
<th>Smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.K.</td>
<td>56 M</td>
<td>Worker in the steel industry</td>
<td>2.16.89</td>
<td>Right upper lobectomy</td>
<td>Positive</td>
</tr>
<tr>
<td>Y.M.</td>
<td>33 M</td>
<td>Welder</td>
<td>10.14.91</td>
<td>cavity in the anterior segment of LUL</td>
<td>Negative¹</td>
</tr>
<tr>
<td>T.T.</td>
<td>72 M</td>
<td>Carpenter</td>
<td>9.28.92</td>
<td>Partial resection of posterior segment of RUL</td>
<td>Positive</td>
</tr>
<tr>
<td>Y.K.</td>
<td>42 F</td>
<td>Housewife</td>
<td>7.26.96</td>
<td>Right upper lobectomy</td>
<td>Positive</td>
</tr>
<tr>
<td>Y.O.</td>
<td>48 M</td>
<td>Worker in the steel industry</td>
<td>7.29.96</td>
<td>Left upper lobectomy</td>
<td>Positive</td>
</tr>
<tr>
<td>J.U.</td>
<td>71 M</td>
<td>Office worker</td>
<td>6.16.98</td>
<td>Right upper lobectomy</td>
<td>Positive</td>
</tr>
<tr>
<td>K.T.</td>
<td>37 M</td>
<td>Office worker</td>
<td>2.24.99</td>
<td>right upper lobectomy</td>
<td>Positive</td>
</tr>
<tr>
<td>S.N.</td>
<td>64 M</td>
<td>volunteer</td>
<td>8.11.99</td>
<td>Left pneumoectomy</td>
<td>Positive</td>
</tr>
<tr>
<td>K.Y.</td>
<td>65 M</td>
<td>Worker in the steel industry</td>
<td>8.6.99</td>
<td>Left lower lobectomy</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Definition of abbreviations: RUL: right upper lobe; LUL: left upper lobe.

*At the time of operation.

¹Culture positive.
Pathological and immunohistochemical findings

Resected lung specimens of each case were stained by hematoxylin and eosin. To evaluate the types of inflammatory cells, immunohistochemical staining was performed, employing the avidin–biotin peroxidase complex method (Dako LSAB kit-peroxidase, DAKO Corp., Kyoto, Japan) using several monoclonal antibodies as follows; anti-CD68 to distinguish monocytes and macrophages (DAKO Corp., 051-M814, 1:100 dilution), anti-α-smooth muscle actin to distinguish smooth muscle and myofibroblasts (DAKO Corp., 093-M851, 1:200 dilution), anti-KL-6 to distinguish epithelial cells (kind gift from Prof. Dr. N. Kohno, Second Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan, 10 μg/ml). In order to retrieve and increase the immunoreactivities, preincubation with 0.1% pronase at 37°C for 20 min was performed for CD68 antibody.

Detection of MAC in pathological specimens

In order to detect MAC, special staining: Ziehl–Neelsen’s method as well as fluorescence method, were performed in several anatomical localizations related to airways.

Results

Pathological findings of bronchiectases

Pathological findings listed below were observed in the airway of all nine patients.

(i) Destruction of bronchial cartilage. Pathologically, extensive infiltration of mononuclear cells, aggregated lymphoid cells, and epithelioid cells was clearly demonstrated in the submucosal layer of the relatively large bronchus which was surrounded by cartilage (Fig. 1A). Bronchial epithelial desquamation and disarrangement of smooth muscle layer were found in these bronchi (Fig. 1A). In the relatively large bronchial wall, destruction with lytic change and interruption of cartilages by granuloma caused by MAC were clearly demonstrated in bronchial ulceration involving their whole walls (Fig. 1B).

(ii) Destruction of smooth muscle layer. Disappearance, disarrangement, or discontinuity of muscle layer by granuloma caused by MAC was revealed by anti-α-smooth muscle actin antibody as shown in Fig. 2. These changes in muscle layer were

Figure 1 (A) Extensive infiltration of mononuclear cells, mainly lymphocytes in the submucosal layer is observed. Note desquamation of epithelium, remaining cartilage and discontinuity of smooth muscle layer. Marked necrotic ulcerative change of bronchial wall is observed. Note remaining partially lytic cartilage (arrow). (B) Higher magnification of lytic cartilages in granuloma caused by MAC are clearly demonstrated. A, B: hematoxylin–eosin staining, A: ×10, B: ×40.

Figure 2 Disappearance of smooth muscle layer compressed by MAC granuloma was revealed by immunohistochemical staining with anti-α-smooth muscle actin antibody. Narrowing of bronchiolar lumen was found, resulting MAC granuloma compression. Hematoxylin–eosin staining, ×10.
very often found in membranous bronchioles (Fig. 2).

(iii) Ulceration of bronchial mucosa. Ulceration of the bronchial mucosa caused by extensive submucosal granuloma was frequently observed associating fragmented or regenerating epithelium (Fig. 3). In some areas, necrotic material was released into the lumen of bronchiole as the cause of airway dissemination of MAC (Fig. 3).

(iv) Narrowing of bronchial lumina. The submucosal multiple granuloma formation compressing the bronchial lumen resulted in the marked narrowing of the bronchiolar lumina (Fig. 4A), as well as the release of necrotic mass or exudative material as shown in Fig. 3. By immunohistochemistry using the anti-KL-6 antibody which detects bronchial epithelium, narrowing of bronchial lumen was clearly seen (Fig. 4B).

Distribution of MAC in relatively large airways

Although MAC was not demonstrated in lesions of bronchiectases using Ziehl–Neelsen’s staining, MAC was demonstrated in granulomas in the lesion of bronchiectasis using a fluorescence microscope (data not shown).

Discussion

In the present study, we demonstrate the pathological findings of bronchiectasis caused by MAC infection in resected lungs. Destruction of cartilage, disarrangement or discontinuity of smooth muscle layer, and ulceration of bronchial mucosa and narrowing of airways were clearly demonstrated. In addition, extensive granuloma formation caused by MAC infection was observed throughout the airways.

It had been believed that the majority of patients with MAC pulmonary disease have chronic underlying lung disease such as pneumoconiosis, bronchiectasis, chronic bronchitis, and emphysema. In these patients, radiographic patterns may be indistinguishable from postprimary tuberculosis, characterized by nodules, consolidation, and cavities, most often affecting the upper lobes and superior segments of the lower lobes.

Prince et al. have reported that pulmonary disease caused by MAC can affect persons without predisposing conditions, particularly elderly women. More recently, there have been several reports describing a strong association between bronchiectasis with circumscribed nodules and MAC.
infections in elderly women in the absence of underlying malignancy or immune compromise. This pattern is characterized by bronchiectasis and nodular densities, particularly affecting the middle lobe and lingula. Hartman et al. suggested retrospectively that the concomitant findings of bronchiectasis and multiple small well-circumscribed lung nodules are indicative of infection or colonization with MAC. In addition, Moore has shown that airway disease in these patients is frequently progressive and have suggested that bronchiectasis may not be a predisposing condition but rather a consequence of atypical mycobacterial infection.

Primack et al. compared HRCT findings in tuberculosis and MAC infection in 77 immunocompetent patients and found that bronchiectasis is significantly more common and more extensive in patients with MAC, identifiable in 94% of the 32 patients compared with 27% of the 45 patients with typical reactivation tuberculosis. Furthermore, the distribution of bronchiectasis varied between the two groups, primarily involving the upper lobes in patients with M. tuberculosis, compared with patients with MAC in whom lobar predominance could not be identified.

Swensen et al., using a combination of HRCT findings of bronchiectasis and small nodules, was able to predict cultures positive for MAC with a sensitivity of 80% a specificity of 87% and an accuracy of 86% in 63 patients with HRCT-diagnosed bronchiectasis in whom mycobacterial cultures were sent. In more recent reports adding on these articles, radiological findings of pulmonary MAC infection has been established.

There are few reports which describe pathological findings of MAC respiratory infection. We have previously evaluated the radiologic and pathologic correlation in patients with MAC. In the present study, we clearly demonstrate the destruction of cartilage and disarrangement or discontinuity of smooth muscle layers. Since cartilage and smooth muscle play an important role in maintaining airway lumen, destruction of these elements could result in bronchiectases. The finding of granulomas is especially significant because this constitutes evidence that bronchiectasis, instead of being a precursor, is likely the result of chronic infection. In addition, peribronchial granuloma formation was observed from the large airway to the bronchiole. In the bronchiole, peribronchial granuloma protruded into the lumen, which resulted in the narrowing of the bronchiole. These pathological findings seem to explain the evidence that obstructive change in the respiratory function test is frequently observed in patients with MAC pulmonary infection. In some areas, necrotic materials were detached into the lumen of bronchiole. This evidence suggests that centrolubal nodules may have been caused by transbronchial dissemination of this necrotic material through the airway. Therefore, in summary, the characteristic pathological finding of pulmonary MAC infection was extensive granuloma formation throughout the airway.

The second purpose of this study was to demonstrate MAC in the lesion of bronchiectases. In the present study, although MAC was not demonstrated in lesions of bronchiectases using Ziehl–Neelsen’s staining, MAC was demonstrated in granulomas in the lesion of bronchiectasis using a fluorescence microscope. Recently, it has been reported that hypersensitivity pneumonitis is caused by MAC especially for users of hot tub. Therefore, to demonstrate MAC in the lesion of bronchiectasis is very important to discriminate MAC infection and hypersensitivity reaction.

In conclusion, in the present report, we clearly demonstrate MAC in the lesion of bronchiectases, suggesting that bronchiectasis was truly caused by MAC infection due to the destruction of bronchial structure.

References


