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Pathological and radiological changes in resected lung specimens in *Mycobacterium avium intracellulare* complex disease

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ABSTRACT: The present study was designed to evaluate the pathological and immunohistochemical findings of *Mycobacterium avium intracellulare* complex (MAC) lung infection.

A retrospective study was performed in five cases with positive cultures for MAC in whom lung resections were performed between January 1989 and December 1996. A determination of whether or not MAC caused pulmonary disease was made using the 1997 criteria defined by the American Thoracic Society. In addition, MAC was cultured from all of the five lung specimens. Pathological and immunohistochemical findings as well as chest computed tomography (CT) findings were evaluated in these five patients.

Pathological findings of bronchiectasis, bronchiolitis, centrilobular lesion, consolidation, cavity wall and nodules were demonstrated, respectively, in relation to chest CT findings. Extensive granuloma formation throughout the airways was clearly demonstrated. Immunohistochemical staining demonstrated: 1) epithelioid cells and giant cells; 2) myofibroblasts extensively infiltrating the cavity wall; and 3) B-cells detected in aggregates in the vicinity of the epithelioid granulomas.

This study identified pathological and immunohistochemical characteristics of *Mycobacterium avium* complex infection relative to chest computed tomography findings and allowed the conclusion that bronchiectasis and bronchiolitis were definitely caused by *Mycobacterium avium* complex infection.

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Pulmonary disease caused by *Mycobacterium avium intracellulare* complex (MAC) in patients without predisposing conditions has become an increasingly common clinical problem [1–5]. Although MOORE [6] reported some pathological findings of atypical mycobacterium, there are few reports comparing pathological findings and radiological findings in patients with MAC infection. In addition, there are no reports which evaluate the type and distribution of inflammatory cells in pulmonary MAC infection. Furthermore, it has been argued whether bronchiectasis is truly caused by MAC infection or is the predisposing condition favouring MAC colonization [6–10]. Based on this background, this study was designed to evaluate the pathological and radiological findings in the lungs of patients undergoing lung surgery for MAC infection and to evaluate immunohistochemically type and distribution of infiltrating cells.

For editorial comments see page 479

Materials and methods

Patients

A retrospective study was performed in five cases with MAC-positive sputum in whom surgical resection of the lung was performed between January 1989 and December 1996. The criteria for defining nontuberculous mycobacteria (NTM) pulmonary disease were those of the American Thoracic Society (1997) [5]. In addition, MAC was cultured from resected lung specimens in all patients. Characteristics of these five patients are listed in table 1. No patient had acquired immune deficiency syndrome (AIDS) or any underlying lung disease. In four of the five patients, there was a history of dust exposure.

Radiological evaluation

Chest computed tomography (CT) (standard CT) was performed in all five patients and chest CT findings were

Table 1. – Patient characteristics

Case No.	Age* yrs	Sex	Occupation	Date of operation	Operation	Smear
1	56	M	Steel industry worker	16/2/89	Right upper lobectomy	Positive
2	33	M	Welder	14/10/91	Partial resection of the cavity in the anterior segment of LUL	Negative
3	72	M	Carpenter	28/9/92	Partial resection of posterior segment of RUL	Positive
4	42	F	Housewife	25/7/96	Right upper lobectomy	Positive
5	48	M	Steel industry worker	29/7/96	Left upper lobectomy	Positive

M: male; F: female; RUL: right upper lobectomy; LUL: left upper lobectomy. *: at the time of operation.

analysed by three radiologists. Chest CT findings were classified as: 1) bronchiectasis; 2) cavity formation; 3) centrilobular nodules (defined as increased attenuation in the area around the terminal or respiratory bronchioles, multiple, well-circumscribed, with the majority of the nodules around 5 mm); 4) consolidation; and 5) nodules (>10 mm). Bronchiectasis was diagnosed when the diameter of the bronchi was larger than that of the accompanying vessels [6].

Pathological and immunohistochemical findings

Resected lung specimens were stained with haematoxylin 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 a panel of monoclonal antibodies (mAbs) identifying specific inflammatory cell types. The mAbs used were: anti-CD68 (1:100) to distinguish monocytes and macrophages (DAKO Corp., 051-M814), anti-UCHL-1 (1:30) to distinguish T-cells (DAKO Corp., 076-M0742), anti-L26 (1:50) to distinguish B-cells (DAKO Corp., 091-M755), anti-α-smooth muscle actin (1:200) to distinguish myofibroblasts (DAKO Corp., 093-M851), anti-KL-6 (10 mg·mL^{-1}) to distinguish epithelial cells (a gift from N. Kohno, Second Department of Internal Medicine, Ehime University School of Medicine, Ehime, Japan), and the polyclonal antibody to S-100 protein (1:800) to distinguish Langerhans and/or dendritic cells (DAKO Corp., 129-Z0311). In order to increase the immunoreactivity, preincubation with 0.1% pronase at 37°C for 20 min was performed before the CD68 antibody staining.

Results

Table 2 shows the preoperative chest CT findings and pathological findings in the resected lung of the five patients. Centrilobular nodules, bronchiectasis, bronchiolitis and cavity formation were observed in all patients. Nodules >10 mm and consolidation were observed in two patients.

The preoperative chest CT findings of bronchiectasis and bronchiolitis are demonstrated in figure 1. Radiologically, the thickening of the walls of large to small airways was clearly demonstrated. Pathologically, extensive peribronchial infiltration of mononuclear cells as well as epithelioid cells, surrounding bronchi (fig. 2a) as well as bronchioles (fig. 2b) was clearly shown. Ulceration of the bronchial wall was frequently observed associated with the derangement or the discontinuity of the muscle layer, as revealed by anti-α-smooth muscle actin staining. In addition, narrowing of bronchioles was frequently observed. In some areas, necrotic material was released into the lumen of the bronchiole. Peribronchial granulomas were observed protruding into the bronchial lumen, resulting in the marked narrowing of the bronchiole (fig. 3).

The preoperative chest CT findings of centrilobular nodules (defined as increased attenuation in the area around

Table 2. – Correlations between preoperative chest computed tomography (CT) findings and pathological findings of the resected lung

Case	Centrilobular	Bronchiectasis	Cavity	Nodules	Bronchiolitis	Consolidation
Preoperative chest CT findings*						
1	Yes	Yes	Yes	No	Yes	Yes
2	Yes	Yes	Yes	Yes	Yes	No
3	Yes	Yes	Yes	No	Yes	No
4	Yes	Yes	Yes	No	Yes	No
5	Yes	Yes	Yes	Yes	Yes	Yes
Pathological findings of the resected lung^{+}						
1	Yes	Yes	Yes	No	Yes	Yes
2	NE	NE‡	Yes	NE	NE	NE
3	Yes	Yes	Yes	No	Yes	No
4	Yes	Yes	Yes	No	Yes	No
5	Yes	Yes	Yes	Yes	Yes	Yes

NE: not evaluable. *: chest CT readings were examined by a radiologist blinded to the pathological reports; $^{+}$: pathological findings were evaluated by two pathologists blinded to the chest CT readings; ‡: NE since only a cavity lesion was resected.

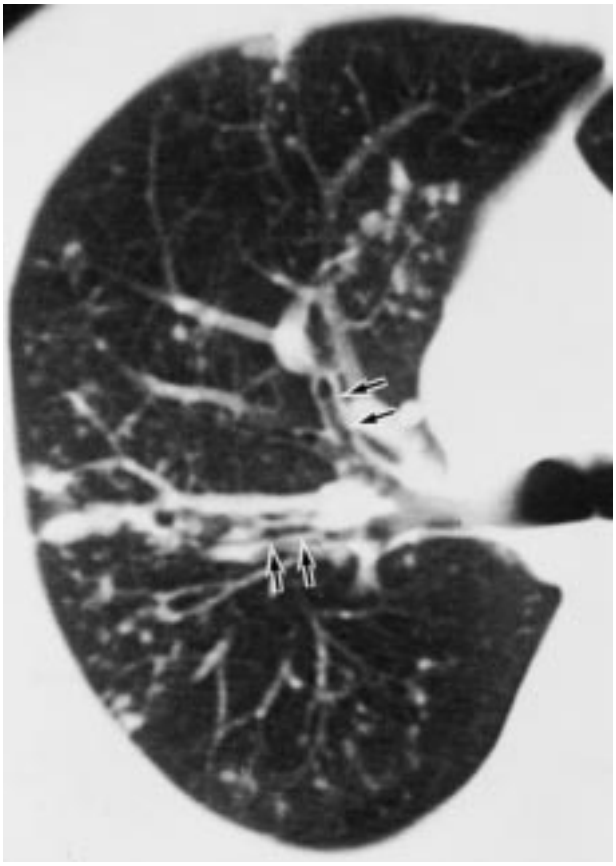

Fig. 1. – Preoperative chest computed tomography findings of bronchiectasis and bronchiolitis. Thickening of the bronchial walls from large to small airways were clearly demonstrated (arrows).

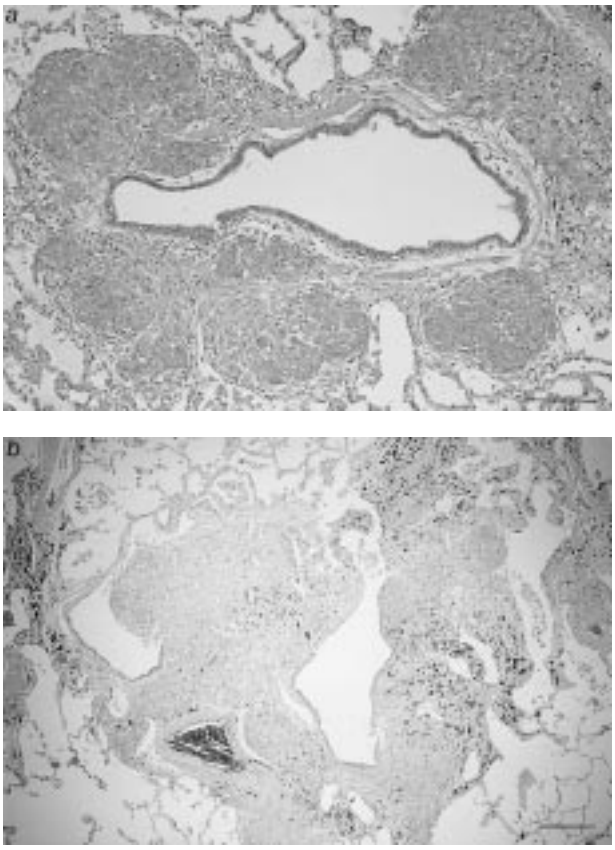

Fig. 2. – Haematoxylin and eosin staining of bronchiectasis and bronchiolitis. extensive peribronchial infiltration of mononuclear cells as well as epithelioid cells, surrounding a) a bronchus or b) a bronchiole, are demonstrated. (Internal scale bar a=800 μm, b=1.5 mm.)

the terminal or respiratory bronchioles, multiple, well-circumscribed, and the majority of nodules ~5 mm) and nodules >10 mm are demonstrated in figure 4. Haematoxylin and eosin staining of these centrilobular nodules showed 2–3 mm discrete granulomas which consisted of mononuclear cells and epithelioid cells (fig. 5). There were also adjacent areas of inflammatory infiltration of the alveolar walls. The pathological examination of nodules >10 mm in diameter demonstrated caseous necrosis in the centre of the granulomas. Immunohistochemical staining for bronchial epithelial cells demonstrated that granuloma formation occurred in the alveolar walls and caused the collapse or the enfolding of several alveolar spaces within the granulomatous lesion. Immunohistochemical evaluation of the cavity wall demonstrated that epithelioid cells were arranged in the peripheral part of the caseous necrosis (fig. 6), and myofibroblasts were extensively found in the outer circumference of the epithelioid cell layer (fig. 7).

Preoperative chest CT findings of consolidation are shown in figure 8. These specimens showed areas of inflammatory thickening of the alveolar walls, loosely grouped granulomas, and/or coalescent inflammatory infiltrates completely replacing the normal alveoli (fig. 9). Oedema in the alveoli adjacent to granulomas was also frequently observed. In some areas, a focal desquamative interstitial pneumonia-like pattern was observed (data not shown).

Immunohistochemical evaluation of the cells in the granuloma demonstrated epithelioid cells and multinucleated giant cells. In addition, aggregates of B-cells were frequently observed in the vicinity of the granuloma. In contrast, T-cells were dispersed in the granuloma as well as in the surrounding tissue. There were few dendritic cells in the granuloma. The immunohistochemical findings on resected lungs are summarized in table 3.

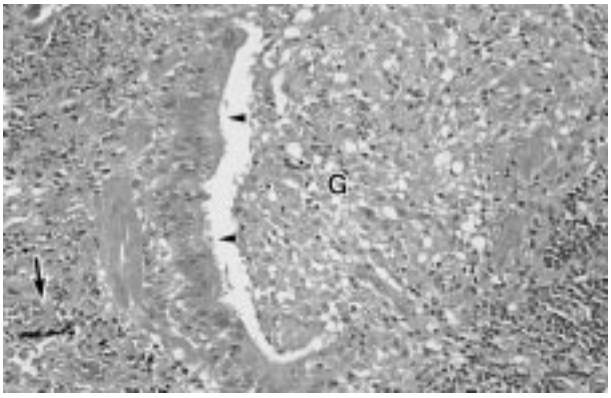

Fig. 3. – Narrowing of bronchiolar air space by a granuloma (G) protruding into the bronchial lumen. Note the covering ciliated epithelial cells (arrowheads). Anthracotic deposits can be observed around this small airway (arrow). (Internal scale bar=50 μm.).

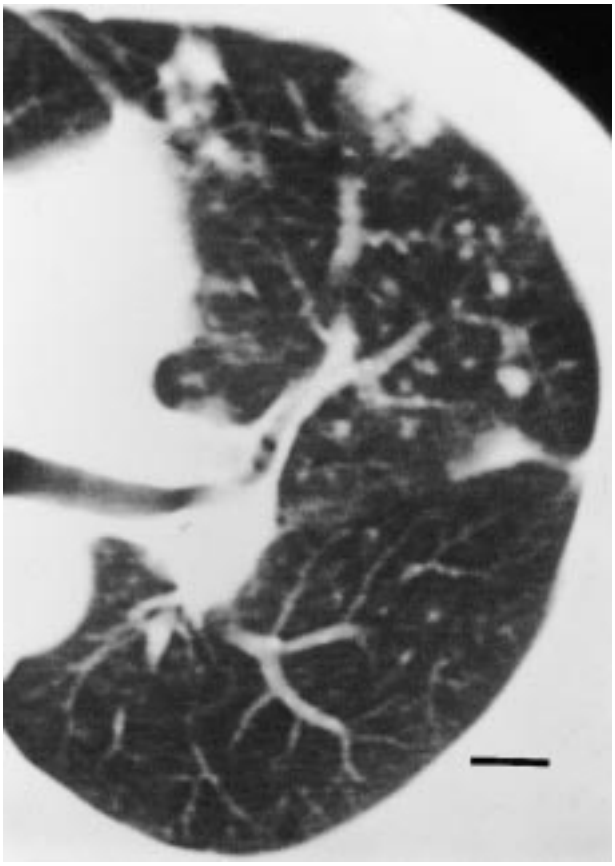

Fig. 4. – Preoperative chest computed tomography findings of centrilobular nodules and nodules >10 mm. (Internal scale bar=1 cm.)

MAC were stained only in the caseating areas of the granuloma. Staining to MAC was positive in four out of the five cases.

Discussion

In the present study, the pathological and radiological findings associated with MAC infection in resected lungs was demonstrated. Extensive granuloma formation caused by MAC infection was observed throughout the lung.

MAC is a ubiquitous pathogen that has been found in soil, domestic tap water and animals [11]. Pulmonary disease caused by MAC infection has a wide spectrum of clinical presentations from simple pneumonic infiltrate to progressive destructive disease [11–13]. Many authors believed that in the majority of patients with MAC pulmonary disease there were chronic underlying lung diseases such as pneumoconiosis, bronchiectasis, chronic bronchitis and emphysema [1, 11]. More recently, however, MAC has increasingly been recognized as a primary infectious agent in immunocompetent patients, especially in females without pre-existing pulmonary disease [1]. Although exposure to MAC often occurs without any clinical manifestation, clinical manifestations may range from no symptoms to signs of destructive or even fatal disease [11]. Interestingly, in the present study, four of the five patients were male and had a history of dust exposure and, consistent with exposure, anthracotic deposits were observed around small airways in the resected lungs.

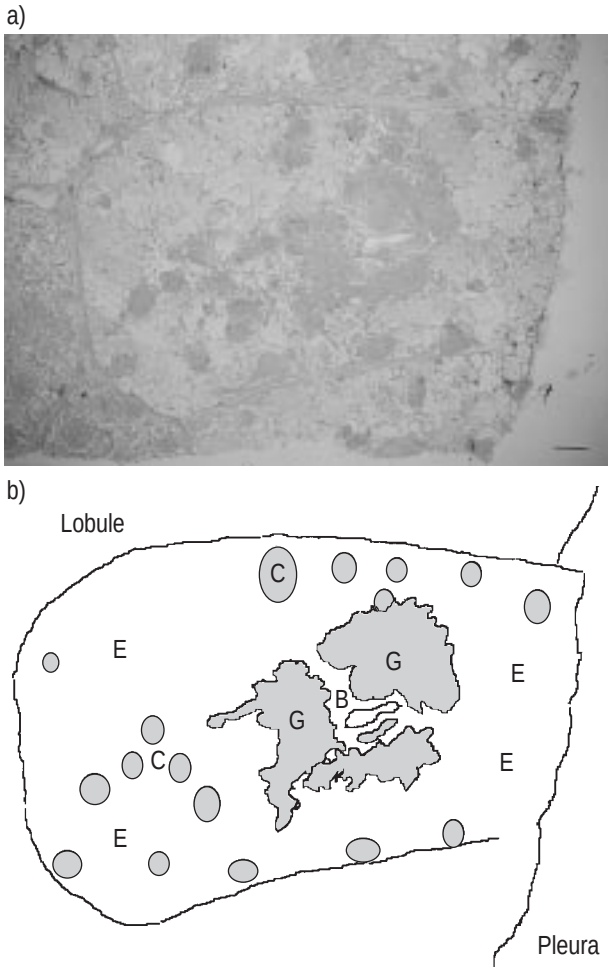

Fig. 5. – a) Haematoxylin and eosin staining of centrilobular nodules in the lobule. b) Diagrammatic representation of (a) showing peribronchial granuloma (G), bronchiole (B), oedema (E) and centrilobular nodules (C). (Internal scale bar=3 mm.)

A number of reports on CT evaluation of patients with MAC have recently been published [6–10]. It has been reported that bronchiectasis with discrete nodules are an important radiological finding of MAC [6–10]. HARTMAN *et al.* [7] suggested that the concomitant findings of

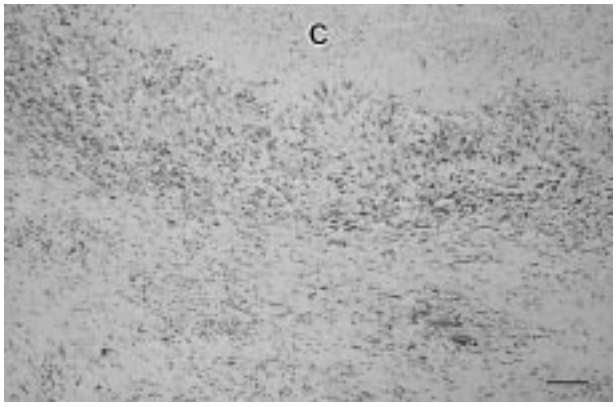

Fig. 6. – Immunohistochemical staining of epithelioid cells in the cavity wall by the anti-CD68 monoclonal antobody. C: the site of caseous necrosis. (Internal scale bar=100 μm.).

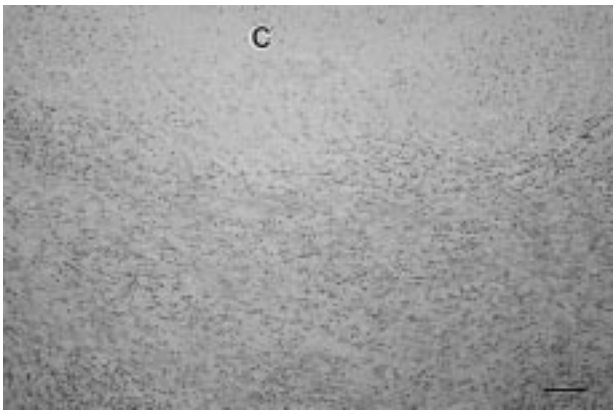

Fig. 7. – Immunohistochemical staining of myofibroblasts in the cavity wall by the anti-α-smooth monoclonal antibody. C: the site of caseous necrosis. (Internal scale bar=100 μm.)

bronchiectasis and multiple small well-circumscribed lung nodules are indicative of infection or colonization with MAC. They identified a subtype of patients (predominantly older females with no underlying malignancy or clinically evident immunodeficiency) with MAC who presented with this characteristic pattern on chest CT scans.

MOORE [6] demonstrated centilobular nodules, bronchiectasis and nodules in a few patients, but no cavitation or consolidation. In the present study, the characteristic pathological finding of pulmonary MAC disease was extensive

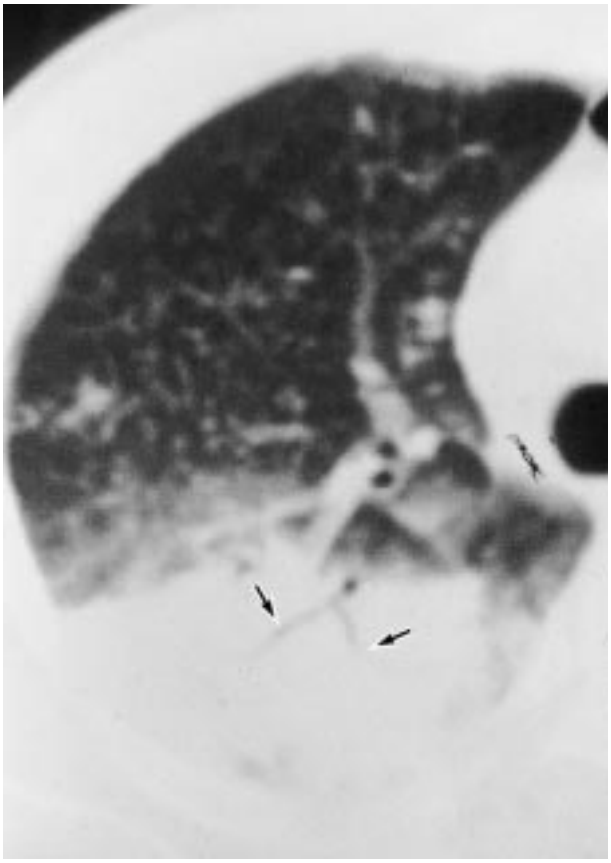

Fig. 8. – The pre-operative computed tomography findings of consolidation. Air-space consolidation and air bronchogram (arrows) are shown.

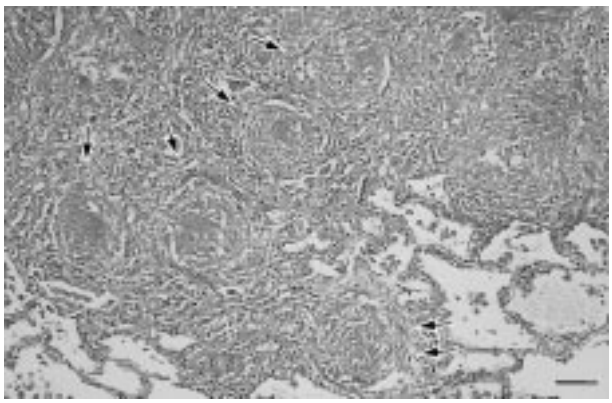

Fig. 9. – Haematoxylin and eosin staining of consolidation. Arrows indicate loosely grouped epithelioid cell granuloma. These granulomas are replacing normal alveoli (double arrow) in association with collapses and enfolding of the alveolar spaces. Inflammatory thickening of the alveolar walls is also seen. (Internal scale bar=100 μm.).

granuloma formation throughout the airways. Peribronchial granuloma formation was observed from the large airway to the bronchiole. In the bronchiole, a peribronchial granuloma protruded into the lumen which resulted in the narrowing of the bronchiole. In addition, ulceration of the bronchial wall with ensuing disruption of the muscle layer was frequently observed, which may have caused bronchiectatic changes. In some areas, necrotic materials were found in the lumen of the bronchiole, suggesting that centrilobular nodules might have been caused by transbronchial dissemination of this necrotic material.

In the air-spaces, loosely grouped granulomas and/or coalescent inflammatory infiltrates completely replaced normal alveoli. Oedema and congestion in alveolar lesions adjacent to the granulomas were frequently observed, suggesting that local circulation in the microvessels and lymphatics were also disturbed by MAC infection. Collapse or enfolding of several alveolar spaces together with focal desquamative interstitial pneumonia may have been partially responsible for these air-space lesions.

In addition, this study identified the major types of inflammatory cells and their distribution in the lesions. In the cavity wall, myofibroblasts were shown to infiltrate the outer wall of epithelioid cells. As it has been reported that transforming growth factor (TGF)-β induces myofibroblasts, TGF-β released from the epithelioid cells may have been responsible for the appearance of myofibroblasts [14]. Interestingly, the distribution patterns of T-cells and B-cells were different. Aggregates of B-cells were frequently observed surrounding the granulomas. In contrast, T-cells were dispersed in and around the granulomas.

In conclusion, although future studies evaluating the differences in pathological and immunohistochemical findings between *Mycobacterium avium intracellulare* complex infection and *Mycobacterium tuberculosis* infection are necessary, this study characterizes the typical lesions of *Mycobacterium avium intracellulare* complex pulmonary infection. The extensive peribronchial granuloma formation along the airways, and also in areas without bronchiectasis and ulcerations of the bronchial wall let us assume that bronchiectasis and bronchiolitis is definitely caused by *Mycobacterium avium intracellulare* complex infection.

Table 3. – Immunohistochemical findings of the resected lung

Antibodies used	Stained cells	Findings
Anti-CD68	Monocytes and macrophages	Epithelioid cells and multinucleated giant cells in granulomas were stained, together with alveolar macrophages
Anti-UCHL-1	pan-T cells	Dispersed T-cells were observed in the granuloma as well as surrounding parts
Anti-L26	pan-B cells	Aggregate B-cells were observed in the vicinity of the granuloma
Anti-α-smooth muscle actin	Myofibroblasts	Myofibroblasts were extensively detected along the cavity wall in the outer circumference of the epithelioid cells
Anti-KL-6	Pulmonary epithelial cells	Collapsed or enfolded alveolar spaces within the granuloma
Anti-S-100	Dendritic cells	Few dendritic cells in the granuloma

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