Pathological Analysis of the Cavitary Wall in Mycobacterium Avium Intracellulare Complex Pulmonary Infection

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Abstract

Objective The present study was designed to evaluate the process of cavity formation in Mycobacterium avium intracellulare complex (MAC) lung infection, pathologically and clinically.

Methods Using resected lung specimens, we first evaluated the distribution of MAC as well as the distribution of myofibroblasts in MAC lung infection according to several pathological findings classified as bronchiectasis, centrilobular nodules, cavity, nodule, bronchiolitis, or consolidation. Resected lung specimens (9 cases) were evaluated by special staining: Ziehl-Neelsen’s method and immunohistochemically for CD68 (stain for monocytes and macrophages) and α-smooth muscle actin (stain for myofibroblasts). Chest CT findings were also examined in these 9 patients. In addition, the serial chest CT scans were reviewed in another 3 patients to evaluate the process of cavity formation, radiologically.

Results Although extensive granuloma formations were observed in every pathological classification, MAC was demonstrated only in the necrotic tissue of the inner surface of the cavitary wall, which was connected to the airway. Myofibroblasts which expressed α-smooth muscle actin were intensely demonstrated in the cavitary wall compared with other pathological classifications. In the cavitary wall, the layer of epithelioid cells and multinucleated giant cells surrounded necrosis, and the layer of myofibroblasts surrounded the layer of epithelioid cells. Chest CT findings demonstrated that the cavitary walls were relatively thick. The evaluation of serial chest CT scans demonstrated that cavities were formed from previously existing nodules.

Conclusions Detection of mycobacteria in the cavitary wall, massive infiltration of myofibroblasts compared with other pathological classifications, and connection to the drainage bronchus, were believed to be important in the process of cavity formation in MAC pulmonary infection.

Key words: Mycobacterium avium intracellulare complex (MAC) infection, cavity formation, pathological and immunohistochemical findings, epithelioid cells, myofibroblasts

Introduction

Pulmonary disease caused by Mycobacterium avium intracellulare complex (MAC) in patients without predisposing conditions has become an increasingly common clinical problem (1–5).

It has been reported that radiological comparisons between MAC and pulmonary tuberculosis demonstrate that MAC has a thin layer cavity compared to that of Mycobacterium tuberculosis (5). In addition, it has also been suggested that the cavity in MAC may be caused by a check-valve mechanism (5, 6).

We have reported that cavity formation is closely related to positive sputum culture in patients with MAC (7). In addition, we evaluated the radiological and pathological findings of MAC infection using 5 resected lung specimens and proved extensive granuloma formation throughout the airway. We also demonstrated the existence of myofibroblasts around the outer zone of epithelioid cells in the cavitary lesion and caseous necrotic granuloma (8).

Since myofibroblasts are believed to have an ability to contract, we hypothesized that myofibroblasts may play an important role in the process of cavity formation. In order to evaluate the process of the cavity formation in MAC, we first evaluated the localization of mycobacteria and myofibroblasts using 9 resected lung specimens (from 9 patients). Chest CT findings were also examined in these 9 patients. In addition, the serial chest CT scans were reviewed in another 3 patients to evaluate the process of cavity formation, radiologically.
Materials and Methods

Patients

A retrospective study was performed in 9 cases of positive sputum cultured for MAC in whom surgical resection of the lung was performed from January 1989 to August 1999. The criteria for defining MAC pulmonary disease were those of the American Thoracic Society (1997) (5). In addition, MAC was cultured from resected lung specimens in 8 of the 9 patients. There were no patients who had AIDS or any underlying lung disease. In 4 of the 9 patients, a history of dust inhalation was obtained. All patients underwent conventional chest CT examination.

Pathological and immunohistochemical findings

Resected lung specimens were stained by hematoxylin & eosin. Pathological findings were classified as i) bronchiectasis, ii) centrilobular nodules (defined as increased attenuation in the area around the terminal or respiratory bronchioles, multiple, well-circumscribed, and the majority of nodules around 5 mm), iii) cavity formation, iv) nodules (larger than 10 mm), v) bronchiolitis, and vi) consolidation (7, 8). The diagnostic criteria for bronchiectasis was as follows: diameter of the bronchus is larger than that of the accompanying vessels (6).

In order to evaluate the distribution of MAC, special staining: Ziehl-Neelsen’s method, was performed in each pathological classification. The avidin-biotin peroxidase complex method (DAKO LSAB kit-peroxidase, DAKO Corp., Kyoto), employing several monoclonal antibodies to detect each marker expressed, was used to evaluate the cell types consisting of granulomas. Monoclonal antibodies used were anti-CD68 to distinguish monocytes and macrophages (DAKO Corp., 1:100 dilution), and anti-α-smooth muscle actin antibody (DAKO Corp., 1:200 dilution) to distinguish myofibroblasts. In order to retrieve and increase the immunoreactivities, preincubation with 0.1% pronase at 37°C for 20 minutes was performed for the CD68 antibody.

Successive radiological evaluation

We also evaluated CT scans taken successively (median interval 17 months) in 25 patients with MAC lung infection. Since new cavity formations were observed in 3 of 25 patients, comparison of chest CT findings before and after the cavity formation was performed.

Results

Extensive granuloma formation throughout the airways was clearly demonstrated. Bronchiectasis, centrilobular nodules, and bronchiolitis were observed in 8 of the 9 patients. Nodules larger than 10 mm in diameter were observed in 4 patients, and consolidation was observed in 5 patients. Cavity formation was observed in all patients.

As for MAC, rod-shaped bacteria were detected only in the caseating necrotic areas of the inner wall of the large cavitary lesion in all cases examined (Table 1). These cavitaries were connected to the airway, and originated from bronchus. The bronchial epithelium was totally destroyed, including the smooth muscle layer. The other 5 parts were divided pathologically (bronchiectasis, centrilobular nodules, nodules, bronchiolitis or consolidation) and were free from bacteria including caseous necrotic lesions of nodular granuloma (Table 1).

Immunohistochemical evaluation of the cavitary wall demonstrated that epithelioid cells intermingling with multinucleated giant cells, which were stained by the anti-CD68 monoclonal antibody, were arranged in the peripheral part of caseous necrosis, and myofibroblasts which expressed α-smooth muscle actin were extensively demonstrated forming bundles in the outer circumference of the epithelioid cell layer (Fig. 1). Although myofibroblasts were demonstrated in granulomas located in bronchiectasis, centrilobular nodules, bronchiolitis, nodules, and consolidation, myofibroblasts were much more intensely demonstrated in the cavitary wall (Table 2).

Examples of chest CT findings in these patients (cases 1–5) are demonstrated in Fig. 2. The cavitary walls were relatively thick in these 5 patients.

Successive follow-up of chest CT in 3 patients with MAC

<table>
<thead>
<tr>
<th>Case</th>
<th>Age &amp; Sex</th>
<th>Bronchiectasis</th>
<th>Centrilobular</th>
<th>Cavity</th>
<th>Nodules</th>
<th>Bronchiolitis</th>
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*NE: not evaluable because the lesion was not included in the resected lung specimen.
Cavity Formation in MAC

Table 2. Distribution of Myofibroblasts in Each Pathological Pattern of Resected Lung

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<thead>
<tr>
<th>Case</th>
<th>Age &amp; Sex</th>
<th>Bronchiectasis</th>
<th>Centrilobular</th>
<th>Cavity</th>
<th>Nodules</th>
<th>Bronchiolitis</th>
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*NE: not evaluable because the lesion was not included in the resected lung specimen. “+”: myofibroblasts were only sparcely observed, “+++”: myofibroblasts are intensively obse: both in the total number and also the number in selected areas.

Figure 1. The immunohistochemical staining of myofibroblasts by the anti-α-smooth muscle actin monoclonal antibody of the cavitary wall (×110). “C” represents the site of caseous necrosis and “L” represents infiltrations of lymphocytes. Note the layer of myofibroblasts (M) surrounding epitheloid cells (E). The layer of epitheloid cells was confirmed by immunohistochemical staining by the anti-CD68 monoclonal antibody (data not shown).

Figure 2. Examples of chest CT finding in the patients in whom surgical resection of the lung was performed (A: case 1, B: case 2, C: case 3, D: case 4, and E: case 5). Note the cavitary walls are relatively thick in these patients.

Figure 3. Examples of chest CT finding in the patients in whom surgical resection of the lung was performed (A: case 1, B: case 2, C: case 3, D: case 4, and E: case 5). Note the cavitary walls are relatively thick in these patients.

Discussion

This is the first report to evaluate the mechanism of cavity formation in MAC pulmonary infection. MAC was found only in the caseating necrotic areas of granuloma located in the cavitary wall, and there was intensive distribution of myofibroblasts around the cavitary wall.

The important finding in the present study is the discrepancy between granuloma formation and distribution of MAC. Although extensive granuloma formation was observed throughout the airways, mycobacteria were demonstrated only in necrotic tissue of the inner surface of the cavitary wall connected to the airway. Since our preliminary data suggested that detection sensitivity of mycobacteria in pathological specimens was not so high, this evidence suggests that the number of MAC was very high in the cavitary wall.

The present study also examined the appearance and distribution of myofibroblasts in different pathologic findings.
Figure 3. Successive follow-up of chest CT in 3 patients with MAC lung infection demonstrated the formation of new cavities (b) from preexisting nodules (a). A: 49-year-old female, B: 51-year-old female, C: 72-year-old female.
Myofibroblasts which expressed α-smooth muscle actin were sparsely demonstrated in granulomas located in bronchiectasis, centrilobular nodules, nodules, bronchiolitis, and consolidation. The presence of myofibroblasts surrounding the granuloma was frequently observed on the outside of epithelioid cells. Importantly, in the cavitary wall, myofibroblasts formed an extensive layer on the outer circumference of epithelioid cells. The role of myofibroblasts in the cavitary wall was unclear. However, these cells might have played a role in: i) encapsulating the MAC in the caseating necrosis from normal lung, ii) discharging the necrotic materials including MAC to the drainage bronchus, and/or iii) shrinking the granuloma to make the granuloma compact. These pathological findings suggest that the process of cavity formation in MAC pulmonary infection was not caused by a check-valve mechanism, but by a discharge of necrotic materials including MAC to the drainage bronchus, possibly by the contraction of the myofibroblast layer. Along with this evidence, successive follow-up of chest CT in 3 patients with MAC lung infection demonstrated the formation of new cavities from preexisting nodules and the existence of the drainage bronchus.

Even in Mycobacterium tuberculosis infection, the mechanisms responsible for liquefaction and cavity formation have never been completely determined (9). Local proteinases (including cathepsin D (9) and matrix metalloproteinases (10)), as well as nucleases and probably lipases, evidently hydrolyze solid caseous material, but what triggers this hydrolysis is unknown. After these enzymes digest solid caseous material, there is an increase in local osmotic pressure which causes fluid to be absorbed, creating an ideal culture medium for the extracellular growth of tubercle bacilli. Because of the high levels of tuberculin-like products present (and probably many other factors), macrophage cannot survive in the liquefied menstruum (9). Therefore, the host is powerless to control bacillary growth in such sites and large numbers of bacilli may be discharged from the cavity into the bronchial tree (9).

In the present study, we revealed a layer of myofibroblasts around the outer zone of epithelioid cells in the cavitary lesion and caseous necrotic granuloma. In addition, we have demonstrated the expression of transforming growth factor (TGF)-β1 (which is a well known cytokine to induce myofibroblasts (11)) in epithelioid cells and multinucleated giant cells (12). These findings suggest that within the infected focus, myofibroblasts differentiated from fibroblasts by TGF-β may play a role by encapsulating MAC and by inhibiting the spread of MAC. Furthermore, it was suggested that the layer of myofibroblasts played a role in the process of cavity formation in MAC pulmonary infection. However, destruction of smooth muscles as well as cartilage around Airways, and digestion of elastic fibers during the process of necrosis seemed to cause the enlargement of the cavity.

Since the present study retrospectively evaluated operated patients with MAC as well as chest CT findings, the process of cavity formation was not fully elucidated. Therefore, prospective studies designed to evaluate the process of cavity formation should be undertaken in the future.

In conclusion, the present study demonstrated that cavities were formed from preexisting nodules. In addition, mycobacteria existed in the cavitary wall and the subsequent infiltration of myofibroblasts around the cavitary wall may have played a role in the process of cavity formation.

References