Demonstration of Calcitonin and Calmodulin by Immunoperoxidase in the Cystic Fibrosis Lung*

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In this investigation, the presence of calcitonin and calmodulin has been demonstrated in immunoperoxidase formalin-fixed, paraffin-embedded sections of lung from autopsy tissues of four patients who died as a result of complications resulting from their cystic fibrosis disease. Immunoreactive calcitonin has been stained and quantitated in solitary endocrine cells which are increased in number and staining intensity in cystic fibrosis lung when compared to COPD and normal lungs. Immunoreactive calcitonin has been demonstrated to be increased in cystic fibrosis lung when compared to COPD and normal lungs. Previously, increased calcitonin and calmodulin were identified in sputum from cystic fibrosis patients utilizing radioimmunoassay. The calcitonin and calmodulin may be associated with increased calcium in pulmonary secretions leading to selective colonization of the lung by a limited number of pathogenic bacteria and enhanced pulmonary infection.

Sputum specimens from patients with cystic fibrosis are colonized by a limited number of bacterial species. Attempts to identify the selective nature of the sputum from cystic fibrosis patients indicate that this selectivity may be due to the ionic composition of the sputum from cystic fibrosis patients. Sputum concentrations of cystic fibrosis patients were analyzed and found to have significant elevations of sodium, potassium, chloride, calcium, magnesium, and iron. In addition, increased concentrations of calcitonin and calmodulin were found in the sputum specimens of the cystic fibrosis patients. These abnormalities may provide the appropriate environment for the colonization by a limited number of bacterial species. Persistent and continuing infection eventually result in the profound pulmonary changes, pulmonary hypertension, and cor pulmonale with cardiac failure. Recently, a new and rare complication of the chronic pulmonary infection in cystic fibrosis patients was reported. One of our patients developed amyloidosis which caused the nephrotic syndrome.

In this investigation, the formalin-fixed, paraffin-embedded autopsy sections of lung from four different patients with cystic fibrosis were studied for the presence of immunoreactive calcitonin and calmodulin by an immunoperoxidase technique.

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Material and Methods

Autopsy sections of lung from four patients with cystic fibrosis were fixed in buffered formalin and paraffin embedded. Control subjects consisted of sections of lung which were histologically normal from patients who died from malignant and infectious illnesses not involving the lungs and from patients with chronic obstructive pulmonary disease (COPD). These control lung sections had also been fixed in buffered formalin and paraffin embedded.

The technique for immunoperoxidase staining for immunoreactive calcitonin and calmodulin was the peroxidase-antiperoxidase (PAP) method to achieve specific staining.

The primary antibody against immunoreactive calcitonin was obtained as was the one against immunoreactive calmodulin. Linking antibody and labeling antibody were utilized. The entire antibody complex was made visible by addition of a chromogenic substrate solution which reacts with the peroxidase label to yield a red to brownish-red stain at the sites of antigen localization in the tissue.

The stained lung tissue slides were evaluated by two different persons. Low power microscopic fields were examined and the percentage of positive cells for calcitonin in the bronchiole epithelium and alveolar pneumocytes was determined by counting the number of cells staining positive for calcitonin per 100 nonstained cells.

Similarly, the amount of reactivity for calmodulin in the bronchiole epithelium and alveolar pneumocytes was determined by estimating the intensity of immunoperoxidase staining for calmodulin. Negative control specimens for both calcitonin and calmodulin were lung tissue samples from patients with COPD and patients who died from malignant and infectious illnesses not involving the lungs. Positive controls for calcitonin were malignant cells of a medullary carcinoma of the thyroid. Positive control specimens for the calmodulin were normal pancreas tissue samples which are known to produce calmodulin.

Results

Positive immunostaining appeared as a red to brownish-red cytoplasmic precipitate. Nuclei did not
stain. Staining intensity was graded 0 to 4+. The intensity of the stain was quantitated as 0, no color; 1+, slight color; 2+, moderate color; 3+, moderate to heavy color; and 4+, heavy to intense color.

The autopsy sections of the lungs from patients with cystic fibrosis who had acute and chronic pneumonitis showed 4+ staining of pulmonary endocrine cells in the bronchiolar mucosa. Each bronchiolar mucosa contained three to eight endocrine cells showing 4+ prominent staining for immunoreactive calcitonin (Fig 1 and Table 1). The number of pulmonary endocrine cells reported in Table 1 is an average of the results of two independent observers. The sections of the control normal lungs and COPD lung showed 0 to 1+ staining for immunoreactive calcitonin in occasional 0 to 1 pulmonary endocrine cells in the bronchiolar mucosa (Table 1 and Fig 2).

Positive immunostaining for immunoreactive calmodulin was obtained in the autopsy sections of lungs from patients with cystic fibrosis with acute and chronic pneumonitis. The staining was 1 to 2+ diffusely in the cytoplasm of almost all the cells in the bronchiolar epithelium and the pneumocytes of the alveoli of the lung sections (Fig 3 and Table 2). The control sections of normal lung and COPD lung showed no staining (0) to faint minimal staining (1+) in the bronchiolar epithelium and alveolar cells (Fig 4).

Table 1—Number of Cells Reactive for Calcitonin per 100 Nonreactive Cells

<table>
<thead>
<tr>
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<th>CF lung tissue Patient 1</th>
<th>CF lung tissue Patient 2</th>
<th>CF lung tissue Patient 3</th>
<th>CF lung tissue Patient 4</th>
<th>COPD lung tissue</th>
<th>Normal lung tissue</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>13-15</td>
<td>10-12</td>
<td>18-20</td>
<td>2-4</td>
<td>0</td>
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</tr>
</tbody>
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Table 2—Average Reactivity of Immunoperoxidase Stain Against Calmodulin

<table>
<thead>
<tr>
<th></th>
<th>CF lung tissue Patient 1</th>
<th>CF lung tissue Patient 2</th>
<th>CF lung tissue Patient 3</th>
<th>CF lung tissue Patient 4</th>
<th>COPD lung tissue</th>
<th>Normal lung tissue</th>
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<tbody>
<tr>
<td></td>
<td>2-3+</td>
<td>1-2+</td>
<td>2-3+</td>
<td>1-2+</td>
<td>0-1+</td>
<td>0-1+</td>
</tr>
</tbody>
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Discussion

Recently, sputum specimens from cystic fibrosis patients were collected and demonstrated increased concentrations of sodium, potassium, chloride, magnesium, calcium, iron, and peptide calcitonin and calmodulin. A summary of the ionic results is reproduced here as Figure 5.1,2 The clinical significance of increased concentrations of some of these ions in the pathophysiology of chronic lung disease has been established. The increased iron is necessary for promotion of bacterial growth.3 The increased magnesium may correlate with the presence of mucoid Pseudomonas aeruginosa.4,5 This mucoid coat enables this organism to resist phagocytosis and digestion by neutrophil leukocytes.6

The increased sodium, potassium, and chloride may be related to a defect in chloride transport in the cystic fibrosis lung.7 The defect also may be due to a decreased sodium reabsorption. The elevated calmodulin and calcitonin in the sputum may be an expression of the genetic defect found in cystic fibrosis. The increased concentrations of these important calcium regulating substances may thus lead to increased calcium in sputum which combined with sputum dehydration causes an increased level of mucus.

Calcium promotes the binding of mucus which promotes the proliferation of mucoid-coated bacterial
species. Calcium channel blockers such as verapamil or nifedipine could lower intracellular calcium levels and prevent mucus thickening and dehydration of sputum. By introducing these agents prior to the onset of lung infection, it may be possible to inhibit infection before it occurs. The calcium channel blockers have been successfully used to treat pulmonary hypertension which may complicate cystic fibrosis with the development of cor pulmonale. Thus, the ionic composition of the cystic fibrosis sputum appears to be a selective environment for the colonization of the cystic fibrosis sputum by only a few bacterial species.

Recently, immunoreactive calcitonin has been localized in solitary endocrine cells and in clusters of these cells called neuroepithelial bodies in human and hamster lungs. It was shown that hyperplasia of pulmonary endocrine cells containing immunoreactive calcitonin occurs following exposure to diethylaminoethylamine, a systemic carcinogen. Pulmonary immunoreactive calcitonin increased significantly at eight weeks following exposure to diethylaminoethylamine. Immunoreactive calcitonin levels in hamster sera and lungs can be utilized as a biochemical parameter to monitor hyperplasia of pulmonary endocrine cells.

Another recent investigation demonstrated that immunoreactive calcitonin was increased in the serum and urine of patients with small cell carcinoma, bronchial carcinoid and inflammatory lung disease. Immunocytochemical stains demonstrated the presence of the hormone in pulmonary Kultchitsky (K cells) cells. Calcitonin has been implicated in pulmonary surfactant secretion. Pulmonary surfactant is synthesized by alveolar type II pneumocytes and stored in inclusions called lamellar bodies. A calcium ionophore (A23187) stimulates lamellar body secretion in vitro with increased amounts of immunoreactive calcitonin production.

The elevated calmodulin and calcitonin present in the autopsy sections of the cystic fibrosis lung correlate with the increased calmodulin and calcitonin in the sputum of the cystic fibrosis patients. The increased calmodulin and calcitonin may be an expression of the genetic defect of cystic fibrosis. Increased calmodulin has been demonstrated in cultured skin fibroblasts from cystic fibrosis patients. Alternately calcitonin and calmodulin may be secondary to increased stimulated production by bacterial infection in the cystic fibrosis lung.

REFERENCES

Occupational Lung Disease and Epidemiology of Respiratory Disease

The Canadian Thoracic Society and the Division of Pulmonary Disease, University of Western Ontario, will sponsor a review course on Occupational Lung Disease and the Epidemiology of Respiratory Disease in Victoria, B.C., July 27-30. For information contact either Dr. William Jeanes, Medical Director, Canadian Thoracic Society, 75 Albert Street, Ottawa K1P 5E7, or Dr. W. K. C. Morgan, University Hospital, 339 Windermere Road, PO Box 5339, Station A, London, Ontario N6A 5A5.


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