

BRIEF REPORT: IDIOPATHIC DIFFUSE HYPERPLASIA OF PULMONARY NEUROENDOCRINE CELLS AND AIRWAYS DISEASE

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IN contrast to fetal lungs, in which neuroendocrine cells are abundant and are thought to play a part in the paracrine regulation of lung development, normal adult lungs contain few neuroendocrine cells.¹⁻⁶ However, neuroendocrine-cell hyperplasia is frequently observed in subjects living at high altitude⁷ and in association with various lung diseases, particularly among cigarette smokers.⁸⁻¹³ Although the role of neuroendocrine cells in these conditions remains unclear, it is speculated that hyperplasia of pulmonary neuroendocrine cells is an adaptive response to hypoxia⁷ or a secondary process associated with chronic lung disease.⁷⁻¹² Recent investigations suggest, however, that alterations in neuroendocrine-cell physiology may precede the onset of clinically detectable lung disease.¹⁴ In addition, neuroendocrine cells elaborate secretions that may affect tissues in their immediate vicinity, such as fibroblasts, epithelial cells, and smooth muscle.¹⁵ Therefore, it is possible that neuroendocrine cells are involved in the pathogenesis of lung diseases.

In support of this concept, we describe six patients with diffuse hyperplasia and dysplasia of pulmonary neuroendocrine cells, multiple carcinoid tumorlets, and peribronchiolar fibrosis obliterating small airways. None of these patients had a history of cigarette smoking or any other concomitant lung disease, yet they had a diffuse hyperplasia of pulmonary neuroen-

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docrine cells severe enough to cause symptoms associated with radiographic and physiologic abnormalities. Furthermore, specific immunostaining for bombesin, a neuropeptide growth factor for human lung fibroblasts,¹³ was demonstrated within these neuroendocrine cells. Thus, it is plausible that the neuroendocrine-cell hyperplasia in these patients may be the primary process and that the airway fibrosis is the result.

CASE REPORTS

A 48-year-old man was seen in consultation for an abnormal chest radiograph that demonstrated diffuse reticulonodular infiltrates without hilar or mediastinal adenopathy. He had never smoked, but he reported more than 20 years of cough. The cough had increased in frequency over the past year and was occasionally productive of clear sputum. He also reported that for five years he had had progressive dyspnea on exertion. His medical history was remarkable only for hemodynamically inconsequential mitral regurgitation, peptic ulcer disease, and bilateral sensorineural hearing loss. His lung examination was normal. A grade 3/6 harsh holosystolic murmur was detected between the left sternal border and apex, without gallop. Clubbing, adenopathy, and edema were absent. The results of a routine laboratory evaluation were normal, as were tests for rheumatoid factors and antinuclear antibodies. Pulmonary-function testing revealed a mixed restriction-obstruction pattern (Table 1). Arterial blood gas analysis and diffusing capacity were normal.

Open-lung biopsy revealed a diffuse hyperplasia and dysplasia of neuroendocrine cells involving distal bronchi and bronchioles, with numerous neuroepithelial bodies present within the mucosa (Fig. 1A). Numerous neuroendocrine-cell tumorlets were also present, of varying size and morphologic features. The smallest lesions involved terminal bronchioles, in which oval to spindle-shaped neuroendocrine cells partially obliterated the walls of these structures and protruded into and partially filled their lumens, as well as the lumens of the associated more distant bronchioles, alveolar ducts, and alveoli (Fig. 1B). These microscopic lesions contained only scant associated fibrosis, but larger lesions, 1 to 3 mm in diameter, were frequently embedded in dense fibroelastic connective tissue. Occasional larger lesions, up to 4 to 5 mm in diameter, revealed circumscribed fibroelastic nodules (Fig. 1C) adjacent to small pulmonary arteries, in which irregularly sized nests of spindled neuroendocrine cells were present; no residual bronchial lumens could be discerned in these lesions. No mitotic figures, areas of necrosis, or nuclear hyperchromatism or pleomorphism were detected. Similar neuroendocrine-cell infiltrates were present focally in the subpleural connective tissue. The pulmonary parenchyma was otherwise unremarkable, with delicate alveolar interstices devoid of inflammation and fibrosis (Fig. 1A and 1B). In electron-microscopical studies, these neuroendocrine cells demonstrated densely stained nucleoli and numerous cytoplasmic neurosecretory granules (not shown). Bombesin-like immunoreactivity and other neuroendocrine immunocytochemical markers were readily demonstrated within these cells with peroxidase immunolabeling procedures described elsewhere.^{13,14} Bombesin-like immunoreactivity and neuron-specific enolase were the most visible markers both within carcinoid tumorlets (Fig. 1D) and in hyperplastic neuroendocrine cells along the epithelium of nonobliterated airways (Fig. 1E). Many of these cells also showed immunoreactivity to chromogranin A and calcitonin. Finally, Movat's pentachrome staining demonstrated a grossly abnormal deposition of disorganized collagen and elastic fibers in both the fibroelastic carcinoid tumorlets and the submucosa underlying areas of neuroendocrine-cell hyperplasia in small airways not obliterated by tumorlets (Fig. 1F).

The patient was treated with two cycles of cisplatin and vincristine without obvious clinical effect. He has remained symptomatically and radiographically stable for eight years, and there is no evidence of metastatic disease. Although cough productive of

Table 1. Clinical Characteristics at Diagnosis of Six Patients with Idiopathic Diffuse Hyperplasia of Pulmonary Neuroendocrine Cells and Airways Disease.

CHARACTERISTIC*	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4	PATIENT 5	PATIENT 6
Age (yr)/sex	48/M	22/M	68/F	61/F	67/F	76/F
Smoker	Never	Never	Never	Never	Never	Never
Cough and exertional dyspnea (yr)†	>20	3	>20	9	15	>20
Pulmonary function						
FEV ₁ (% of predicted)	70	77	33	51	33	35
FVC (% of predicted)	66	69	72	61	60	48
FEV ₁ /FVC (%)	76	87	45	68	40	49
TLC (% of predicted)	84	64	111	92	81	147
RV (% of predicted)	85	54	128	147	92	173
DLCO (% of predicted)	119	121	61	—	73	73
A-aO ₂ (mm Hg)						
Resting	13	1	13	—	12	—
Exercise	30	3	15	—	—	—
Reticulonodular infiltrates on chest film	Yes	Yes	No‡	Yes	Yes	Yes

*FEV₁ denotes forced expiratory volume in one second, FVC forced vital capacity, TLC total lung capacity, RV residual volume, DLCO diffusing capacity for carbon monoxide, and A-aO₂ alveolar–arterial oxygen gradient.

†Except for Patient 6, who had lung crackles, all the patients had normal lung examinations.

‡A chest CT scan in this patient demonstrated diffuse peribronchial thickening and hyperlucent areas suggestive of air trapping.

scanty clear sputum has persisted, the results of serial pulmonary-function tests have remained stable, with only a mild increase in airflow obstruction.

The second patient was a 22-year-old man who presented with progressive dyspnea on exertion and nonproductive cough of approximately three years' duration. He had never smoked. There was no family history of lung disease. Physical examination and routine laboratory evaluation were normal. The chest radiograph showed diffuse reticulonodular infiltrates without pleural thickening or mediastinal adenopathy. Pulmonary-function testing revealed a mild restrictive ventilatory defect with maintenance of normal gas exchange (Table 1). Open-lung biopsy revealed histologic abnormalities, bombesin-like immunoreactivity, and pentachrome staining identical to those of the first patient. The patient was treated with two cycles of fluorouracil and streptozocin without obvious benefit. He remained clinically stable over the next three years, but in the fourth year after the biopsy (seven years after the onset of symptoms), there was radiographic evidence of disease progression accompanied by increased dyspnea. He was then treated with fluorouracil alone, again without response. Although there was never evidence of extrapulmonary metastasis, progressive lung disease and progressive hypoxemia continued to develop, and the patient subsequently died of respiratory failure. An autopsy was not performed.

We studied four additional patients who had the same histopathologic and immunohistochemical features on open-lung biopsy (Table 1). All were elderly women who reported having nonproductive cough and dyspnea for many years. They presented with mild-to-severe airflow obstruction despite never having smoked cigarettes. Three patients had normal lung examinations, and one had bibasilar rales. Three had diffuse reticulonodular infiltrates on chest radiography, but one had a normal chest radiograph. The diffusing capacity was reduced in three patients, but all had normal antiprotease levels. The patient with the normal chest radiograph had a high-resolution chest CT scan that demonstrated diffuse peribronchial thickening and multiple hyperlucent areas suggestive of air trapping. One other patient had a high-resolution chest CT scan that demonstrated multiple small nodules (<10 mm) in all five lobes, with diffuse bronchial-wall thickening and hyperlucent areas suggestive of air trapping. The patient with the normal chest radiograph had a thoracotomy for diagnosis of this disease, but the other three patients had thoracotomies for resection of pulmonary nodules that ranged from 1 to

3 cm in diameter. These were all diagnosed as carcinoid tumors. One of these patients had two thoracotomies, four years apart, for the resection of multiple carcinoid tumors. None of these patients received chemotherapy. Two patients had partially reversible airflow obstruction. All the patients have been treated with inhaled bronchodilators, without relief of their cough and dyspnea. All four patients have remained stable, with an average follow-up period of seven years. A thorough search of the medical literature yielded only one additional case with similar clinical and histopathologic features.¹⁶

DISCUSSION

The concept of a diffuse neuroendocrine system was introduced by Feyrer in 1938 when he hypothesized that epithelial cells with characteristic morphologic and histochemical properties could have important paracrine regulatory functions.¹⁷ A decade later, Frölich published the first description of bronchial neuroendocrine cells,¹⁸ and since then numerous investigators have studied the structure and distribution of pulmonary neuroendocrine cells, as well as their secretory products.^{1,19} However, skepticism has prevailed about the pathophysiologic relevance of pulmonary neuroendocrine cells.

In the earliest descriptions of abnormal tumorlike clusters of pulmonary neuroendocrine cells, the so-called carcinoid tumorlets were thought to represent subclinical *in situ* carcinoma.²⁰⁻²⁴ However, it became apparent that chronic lung scarring, particularly bronchiectasis, was frequently associated with carcinoid tumorlets and that the clinical course of these patients was primarily determined by the underlying scarring lung disease.^{25,26} Further observations led to the concept that carcinoid tumorlets originate from hyperplastic neuroendocrine cells within the airway epithelium,²⁷⁻²⁹ and the possibility was considered that carcinoid tumorlets were inducing a fibrotic reaction.²⁹ More recently, hyperplasia of pulmonary neuroendocrine cells, with or without carcinoid tumorlets, has been described in various lung diseases, such as bronchopulmonary dysplasia,⁸ cystic fibrosis,⁹ asthma,¹⁰ diffuse panbronchiolitis,¹¹ chronic obstructive pulmonary disease,¹² and eosinophilic granuloma.¹³ In each instance, however, hyperplasia is regarded largely as a secondary tissue reaction, and in only a few cases has a pathogenetic role been entertained for neuroendocrine cells and neuropeptides.^{13-15,29,30}

Although we have no direct evidence of cause and effect, we believe that the cases described support our hypothesis that hyperplastic neuroendocrine cells may cause airways disease. The histopathologic pattern was consistent in all patients: a heterogeneous spectrum of lesions, ranging from diffuse hyperplasia and dysplasia of solitary neuroendocrine cells to numerous neuroepithelial bodies, prominent carcinoid tumorlets, and even typical carcinoid tumors. These lesions were frequently associated with airway-wall thickening and fibrosis, but there were no other histopathologic lesions, and there was only minimal or no inflammation. Most important, none of these patients smoked cigarettes or had any other concomitant lung disease. Hence, it is difficult to consider their neuroendocrine-cell hyperplasia a secondary process.

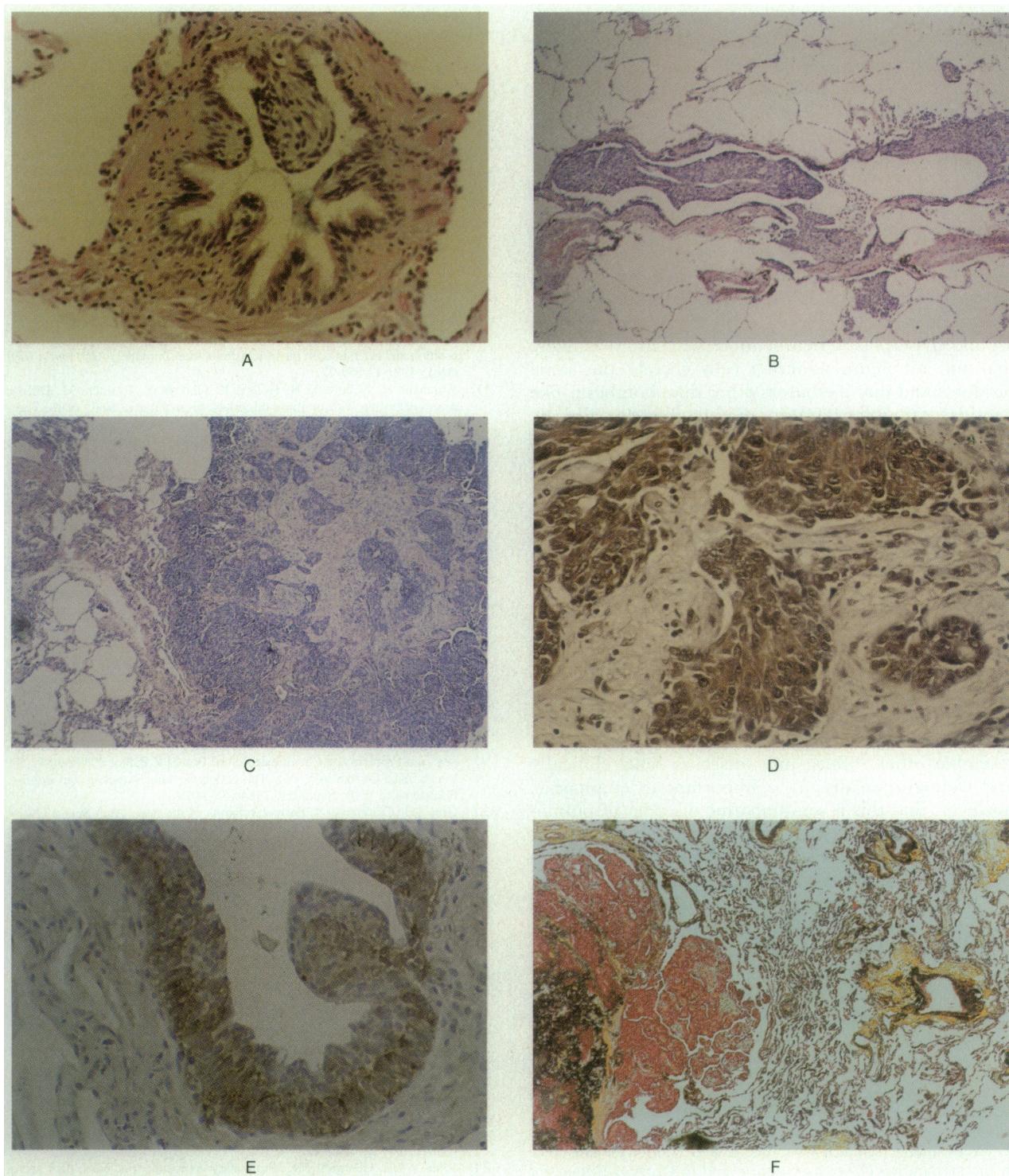


Figure 1. Open-Lung-Biopsy Specimens from Patient 1.

Panel A shows a neuroepithelial body within the bronchiolar mucosa (hematoxylin–eosin, $\times 340$). Panel B shows a neuroendocrine-cell cluster obliterating the walls of a terminal bronchiole and protruding into the lumen of associated air spaces (hematoxylin–eosin, $\times 110$). Overall, the interstitium remains normal and devoid of inflammatory infiltrates or fibrosis. Panel C shows a well-circumscribed fibroelastic nodule with several irregularly sized neuroendocrine-cell nests (hematoxylin–eosin, $\times 60$). No residual lumens could be discerned in these larger lesions. Panel D shows neuroendocrine-cell tumorlets with bombesin-like immunoreactivity (brown areas) embedded within dense fibroelastic tissue (immunoperoxidase, $\times 340$). The residual airway lumens can no longer be discerned. Panel E shows bombesin-like immunoreactivity (brown areas) within an area of linear neuroendocrine-cell hyperplasia in a nonobliterated airway (immunoperoxidase, $\times 350$). A pedunculated cluster of neuroendocrine cells can be seen. Panel F shows a panoramic view of the biopsy specimen, demonstrating grossly abnormal deposition of elastin (black) and collagen (yellow) fibers, both within the carcinoid tumorlets and around the small airways that are not obliterated but that are affected by diffuse neuroendocrine-cell hyperplasia (Movat's pentachrome, $\times 60$).

The intervening interstitium is normal.

All the patients reported cough and exertional dyspnea for many years, and all but one of them presented with diffuse reticulonodular infiltrates on chest radiographs. The most common physiologic abnormality was irreversible airflow obstruction, which may have been caused by a combination of intraluminal obstruction by hyperplastic neuroendocrine cells and peribronchiolar fibrosis (Fig. 1). Nonetheless, the secretagogue and bronchoconstrictive effects of bombesin-like peptides may also play a part,^{31,32} particularly in patients with partially reversible airflow obstruction. In addition to the airways disease, two patients presented with a reduction in lung volumes that appeared to reflect a more severe degree of fibrosis around hyperplastic neuroendocrine cells. It is likely that not all neuroendocrine cells secrete the same products and that mediators other than bombesin-like peptides are important in modulating this fibrotic process. It is also important to note that although five of these patients had relatively benign courses with many years of symptoms and longstanding radiographic changes that showed little progression, one patient had relatively rapid progression of disease and died of respiratory failure seven years after symptoms developed. Thus, not only the pulmonary-function abnormalities but also the course of this disease may be variable. Finally, in some cases a similar histopathologic process may evolve into an overtly neoplastic disorder accompanied by metastasis.^{33,34} It remains uncertain, however, whether these are case reports of the same disease.

In summary, we describe a distinct entity that may resemble other diffuse lung diseases both clinically and radiographically. It is important to emphasize, however, that this is an idiopathic disorder of pulmonary neuroendocrine cells in the absence of other concomitant lung diseases. We suggest that whereas this is an unusual clinical entity, our observations support the concept that hyperplastic neuroendocrine cells may cause airway fibrosis. Therefore, it is possible that hyperplastic neuroendocrine cells are involved in the pathogenesis of bronchopulmonary dysplasia,^{8,30} chronic obstructive pulmonary disease,¹² and other common airway disorders^{7,10,11,13,28} that are characterized by both airway fibrosis and neuroendocrine-cell hyperplasia.

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