Interstitial Pulmonary Fibrosis and von Recklinghausen’s Disease. An Ultrastructural and Immunofluorescent Study*

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We present the clinical and pathologic findings in a 28-year-old white woman with pulmonary fibrosis and von Recklinghausen’s disease. Ultrastructural examination of a lung biopsy specimen showed increased collagen in the alveolar wall, associated with hyperplasia of granular pneumocytes. Large numbers of intraalveolar cells, morphologically suggestive of macrophages, but having tight junctions similar to epithelial cells were also present. Direct immunofluorescent examination using goat anti IgG, IgM, IgA, complement, albumin, and fibrinogen failed to show specific fluorescence. Despite certain structural similarities to other familial and idiopathic forms of interstitial pneumonia, the pathogenesis remains poorly understood.

Massaro et al,¹ Israel-Asselain et al,² and Massaro and Katz³ recently documented 22 cases of pulmonary fibrosis in patients with von Recklinghausen’s disease. Three additional cases have been reported from France⁴,⁵ and Great Britain.⁶ Men far outnumber women; however, the largest number of cases have come from Veterans Administration hospitals.³ Coexisting von Recklinghausen’s disease and pulmonary fibrosis have also been reported in women,⁵,⁶ as well as in a mother and son.²

The etiology and pathogenesis of interstitial pulmonary fibrosis associated with von Recklinghausen’s disease is unknown. The present report presents the first case studied by electron microscopy and immunofluorescent techniques.

CASE REPORT

A 28-year-old white woman was admitted for the first time to Thomas Jefferson University Hospital on Sept 11, 1972, because of dyspnea and abnormal findings on a chest roentgenogram. Her mother had von Recklinghausen’s disease. Since childhood the patient had had numerous cutaneous neurofibromas and cafe au lait spots, which increased in size and number over the past five to eight years. A seizure disorder classified as either psychomotor or grand mal epilepsy had been present for 12 years, for which she had taken diphenylhydantoin, 100 mg, four times a day intermittently for the past three years. Gradually increasing exertional dyspnea over the past two years progressed to the point at which she became short of breath after walking one block or climbing one flight of stairs. Cough, productive of small quantities of yellow sputum was present for about one year.

A chest roentgenogram was reported as within normal limits in June, 1972. A subsequent film taken in September, at Jefferson Hospital, showed mild diffuse interstitial infiltrations suggestive of fibrosis, in both lower lobes. The right apex showed linear fibrosis, but no bullae (Fig 1).

Physical examination on admission to the hospital showed normal vital signs and blood pressure. Multiple soft, pedunculated neurofibromas were present on the scalp, face, arms, legs, back, chest, breast and abdomen. Café au lait spots were present over both arms and chest. Examination of the heart and lungs showed no abnormalities. Neurologic examination showed mild left hemiparesis and slightly increased deep tendon reflexes in the left arm and leg. Clubbing of the fingers and toes was present without cyanosis. Results of laboratory studies for the hemoglobin, hematocrit, white blood cell count, sedimentation rate and protein electrophoresis were within normal limits. Sputum cultures showed

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normal flora. Three lupus erythematosis preparations, fluorescent antinuclear antibody titer and the latex flocculation test were negative for pathologic findings. The serum compliment was 110 mg percent (normal 124 to 188 percent). The serum 1 antitrypsin activity was normal. The results of the pulmonary function tests are seen in Table 1.

On Sept 28, 1972, an open lung biopsy was performed. The course was uneventful after operation, and she was discharged taking diphenhydantoin, 100 mg, three times a day.

Pathology

The alveolar septae showed mild focal thickening associated with sparse interstitial infiltration by inflammatory cells, chiefly lymphocytes, and large mononuclear cells, with occasional polymorphonuclear leukocytes and rarer eosinophils. Interstitial smooth muscle and fibrous tissue were mildly increased in focal areas of alveolar wall thickening, mainly in the subpleural regions of the biopsy specimen. Subpleural cyst formation was present, which appeared to form from disruption and rupture of thickened and clubbed alveolar septae (Fig 2). Such areas were seen in approximately 5 to 10 percent of the biopsy section surface area. The deeper lung parenchyma showed little alveolar wall thickening, and alveolar spaces contained many large eosinophilic cells (Fig 3). These had enlarged irregular nuclei and foamy cytoplasm that contained finely scattered yellow-brown pigment. The intraalveolar cells stained positively with periodic acid-Schiff (PAS), which persisted after diastase digestion. The cells lining the alveoli were prominent, and PAS-negative. When stained for iron with the Prussian blue reaction, scattered foci of intraalveolar cells showed finely granular iron pigment, while others did not stain. The numbers of intraalveolar cells were greatest in regions showing little or no alveolar wall thickening, and were less frequent in the subpleural zones showing cystic changes and fibrosis. These areas of intra-alveolar cellular exudation were seen in 40 to 50 percent of the biopsy-specimen surface area. The remainder of the lung appeared normal. The bronchi, bronchioles and pulmonary vessels showed no abnormality. Infiltration of bronchi and blood vessels by inflammatory cells was not seen, and nodular collections of lymphocytes were not present. Examination under polarized light failed to demonstrate birefringent crystals.

Table 1—Results of Pulmonary Function Tests in Patient with Pulmonary Fibrosis and von Recklinghausen’s Disease

<table>
<thead>
<tr>
<th></th>
<th>Actual, Percent</th>
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<tbody>
<tr>
<td>FVC (ml)</td>
<td>2,211 77</td>
</tr>
<tr>
<td>FEV1</td>
<td>1,769 80</td>
</tr>
<tr>
<td>Max. vol. vent. liters/M</td>
<td>70 85</td>
</tr>
<tr>
<td>Max. Exp. Flow Rate liters/M</td>
<td>175</td>
</tr>
<tr>
<td>Max. Mid. Exp. Flow Rate liters/M</td>
<td>112</td>
</tr>
<tr>
<td>Dlco (single breath) = 7 ml/min/mm Hg</td>
<td></td>
</tr>
<tr>
<td>FRC = 3.29 liters</td>
<td></td>
</tr>
<tr>
<td>RA at FRC = 2.175 cm H2O/liters/sec</td>
<td></td>
</tr>
</tbody>
</table>

Arterial Blood Gas Values

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2 Sat. %</td>
<td>93.4</td>
<td>90.1</td>
</tr>
<tr>
<td>Po2 mm Hg</td>
<td>66</td>
<td>58</td>
</tr>
<tr>
<td>PCO2 mm Hg</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>pH</td>
<td>7.43</td>
<td>7.43</td>
</tr>
</tbody>
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Figure 1. Diffuse bilateral interstitial changes are seen at both bases, slightly more prominent at right. Linear stranding is seen in right apex, but no definite bullae.

Figure 2. Alveolar septae are slightly thickened and sparsely infiltrated by lymphocytes. Septae are ruptured, with early cystic transformation. Intraalveolar mononuclear cells are easily seen (H & E x 50).

Figure 3. Numerous large intraalveolar cells are present. Alveolar septae show no inflammation (H & E x 200).
week. Tissue was postfixed in osmium tetroxide and embedded in a synthetic resin (Epon). Thick sections were stained with toluidine-blue, and selected blocks were stained for electron microscopy with uranyl acetate and lead citrate. An RCA EM U 4A electron microscope was used for examination. Alterations corresponding to those seen on light microscopy were present in all sections examined ultrastructurally. The interstitium between alveolar spaces was thickened by increased numbers of mature collagen fibers. Focal increase in elastic fibers was also seen. Large intraseptal macrophages with numerous membrane-bound lysosomes, as well as mast cells and rare polymorphonuclear leukocytes were present in the thickened septae. The endothelial cell cytoplasm of septal capillaries appeared swollen. There was no alteration of the epithelial or endothelial basement membranes, which remained distinct and homogeneous. Membranous pneumocytes were infrequently seen lining the alveoli. Approximately 80 to 90 percent of the cells attached to the alveolar basement membrane were granular pneumocytes, having electron dense cytoplasm and short microvilli at their free borders (Fig 4). The cytoplasm contained characteristic laminated bodies, which were concentrated toward the luminal border of the cells. Approximately one half of the granular pneumocytes showed prominent dilatation of rough endoplasmic reticulum, which ramified throughout the cell. These cells also showed increased numbers of swollen mitochondria, and together these structures filled most of the cytoplasm (Fig 5). Golgi apparatus were difficult to delineate in these cells. The remainder of the electron-dense cells displayed prominent Golgi apparatus and rough endoplasmic reticulum, which showed only focal cisternal dilatation. Both varieties of granular pneumocytes showed frequent electron dense membrane-bound lysosome-like bodies and aggregated glycogen granules. A less frequently identified light cell was present on the basement membrane, often interspersed between the darker cells, and accounting for approximately 5 percent of the cells attached to the alveolar basement membrane. These were less electron dense and contained numerous laminated bodies dispersed throughout the cytoplasm. The microvilli at the luminal aspect were similar but less numerous than those of the darker cells. The cytoplasm contained numerous ribosomes, both free and bound to the endoplasmic reticulum (Fig 6). Golgi apparatus were easily identified, as were glycogen granules and coated vesicles. Both the light and dark cells were attached to one another by tight junctions and appeared to be variants of granular pneumocytes.

The great majority of cells within the alveolar space had the general appearance of macrophages. Rare granular pneumocytes were also present. The cytoplasm of the macrophages did not contain lamellar bodies and the free borders showed irregular pseudopodia, unlike the microvilli of the granular pneumocytes. The cytoplasm showed numerous irregular electron dense bodies within vesicles, consistent with lysosomes containing lipofuscin and lipid material. Occasionally these cells showed tight junctions when in apposition to one another or with granular pneumocytes (Fig 7). The alveolar space sometimes contained cellular debris and free laminated bodies. No fibrin was seen. Crystalline material suggestive of viral particles was not identified in the cytoplasm or nucleus. Foreign crystalline material was not observed.

**Immunofluorescent Studies**

Quick frozen sections of the lung biopsy section were cut at 6 μ and stored at −20°C for two weeks. They were then
stained with fluorescein conjugated goat antihuman IgG, IgM, IgA, complement, fibrinogen, and albumin (Hyland Laboratories), by the direct technique. No specific fluorescence was observed. Culture of the frozen lung tissue was negative for viruses.

**DISCUSSION**

This patient had signs of von Recklinghausen's disease since childhood, with pulmonary symptoms characterized by shortness of breath and severe diffusion impairment at age 28. The coexistence of von Recklinghausen's disease and pulmonary fibrosis has been reported in patients ranging from 34 to 70 years of age, with a median of 46 years. The physiologic abnormalities exhibited in this case were otherwise similar to previous reports. Most cases have shown diffuse, bilateral interstitial infiltrates suggesting pulmonary fibrosis, roentgenographically. In addition, apical bullae were reported in 19 of 20 patients by Massaro and Katz, but eight had no evidence of disease elsewhere in the lungs. In the absence of physiologic data, differentiation of those patients with only apical changes from bullous emphysema can only be conjectured. Nevertheless, even if these eight cases are discarded, histologic or roentgenographic evidence of diffuse pulmonary fibrosis and alveolitis was present in over 12 percent of patients with neurofibromatosis examined in that study. The paucity of other scattered case reports suggests that the incidence of this association is actually considerably lower, and patients selectively admitted to Veterans Administration hospitals primarily because of lung disease accounted for a significant number of cases in their report. No cases of pulmonary fibrosis were encountered in the extensive study of 223 unselected cases of neurofibromatosis by Crowe et al.

An extraneous etiology of the lung disease in the current case could not be ascertained. The sporadic administration of diphenylhydantoin is not likely to be a significant factor. No evidence of hilar adenopathy or lupus erythematosus complicating diphenylhydantoin therapy was present. Interstitial pulmonary fibrosis from this drug has been suggested, but not generally accepted.

The majority of cases previously reported have shown severe interstitial fibrosis of the lung with cystic honeycombing, indistinguishable from end-stage fibrosing alveolitis. Pathologically, the lung disease in our case was characterized by large numbers of intraalveolar eosinophilic cells, and the septae showed hyperplasia of alveolar lining cells with mild fibrosis and alveolitis. Subpleural cystic changes with alveolar rupture were also present. At the ultrastructural level, granular pneumocytes with prominent lamellar bodies lining the alveoli and large

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**FIGURE 6.** Light granular pneumocyte (G) is seen along epithelial basement membrane (arrow). Laminated bodies and cytoplasmic organelles are less numerous than in adjacent darker cells. Intersitium contains abundant collagen (C). Two macrophages (M) and septal plasma cell (P) are present in intersitium. Swollen capillary endothelial cell (E) is seen in upper left (mag. × 6,180).

**FIGURE 7.** Numerous macrophages (M) are present in alveolar space. These demonstrate pseudopod-like projections and numerous irregularly shaped dense lysosomes, probably containing lipid material. Granular pneumocytes (G) are attached to alveolar basement membrane (arrows). Tight junctions (T) are observed between granular pneumocytes and macrophages (mag. × 5,850).
numbers of cells having the characteristics of intra-alveolar macrophages were significant features. Increased numbers of collagen fibers were readily observed in the interstitium. The light microscopic changes were similar to those previously described in patients with von Recklinghausen's disease. No previous electron micrographs have been published. Variations in the granular pneumocytes similar to that seen in the present case have been observed in cases of desquamative interstitial pneumonia (DIP) reported by Farr, et al and Shortland et al. Septal collagen deposition and the presence of intraalveolar macrophages have also been reported. Indeed, a case suggestive of DIP, on light microscopy, associated with von Recklinghausen's disease has been previously observed. The cell membranes of the intraalveolar cells were of extreme interest. The presence of tight junctions associated with cells otherwise having the characteristics of macrophages suggest possible transformation of the granular pneumocytes. However, this could not be definitely proved from our observations. While tight junctions and desmosomes are characteristic features of epithelial cells, desmosomes and hemidesmosomes have been described in diverse mesenchymal tissues and rat peritoneal macrophages. Suzuki et al have recently observed transformation of alveolar lining cells into active phagocytes with many morphologic characteristics of alveolar macrophages.

Despite our observations, the pathogenesis of the disease process remains unknown. Interstitial fibrosis and cyst formation may be the expression of an inherited mesenchymal defect which results in the primary deposition of collagen, rather than a postinflammatory reparative reaction. Fibrous tissue proliferation in diverse mesenchymal tissues, such as blood vessels and bone, have been infrequently described in von Recklinghausen's disease, lending some support to this view.

The immunofluorescent methods contribute to the understanding of the pathogenesis of the lung disease only in the sense that circulating antibody was not demonstrated present in the lung at the stage of disease that the biopsy was performed. The absence of fixed globulins or compliment and of circulating antitoxins, however, minimizes their possible etiologic role.

The growing number of reports of familial interstitial fibrosis demonstrates that pulmonary fibrosis can be genetically transmitted. Although the pathogenesis of the inherited disease is not understood, vertical transmission invites comparison to those patients with von Recklinghausen's disease. One instance of familial pulmonary fibrosis and neurofibromatosis has been reported. Although there may be possible common factors relating pulmonary fibrosis with von Recklinghausen's disease to other types of interstitial pneumonia, our studies have failed to firmly establish a relationship. The ultrastructural similarities to those reported in some cases of DIP may reflect a limited reaction pattern of the lung to injury, rather than a similar etiology or pathogenesis.

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Home, Sweet Home

Had the Trojan prophetess, Cassandra, been living in our times, she would have, like in ancient days, encountered much public incredulity when announcing that there were 27,500 deaths from home accidents (falls, fire and flames, poisonings, aspiration and ingestion of food, mechanical suffocation, etc) in the United States in 1971. In addition to catastrophic events on which these data are based, there are a great many other circumstances representing serious health hazards in homes. From infancy to old age, allergy in members of the family is one of the most common factors which may set the stage for episodes of sickness or sometimes, of alarming illness. Even though adverse reactions of some individuals to certain foods have been known since the early days of recorded history and as early as 1717 Floyer (A Treatise on the Asthma, London, Wilkins, 1717) attributed asthma to food intake, its high incidence is still with us and the pathologic mechanism of food allergy remains poorly understood. In connection with the long list of food allergens, it is of interest to recall that there are some 2,000 varieties of potatoes. Their putative origin is in the South American Andean plateau. Potato, Solanum tuberosum, belongs in the family of Solanaceae which includes also belladonna, henbane, Jimson weed, pepper, petunia, tobacco and tomato. The latter is an important ingredient of barbecue sauce. The Mayans used sweet potato, tomato, allspice, cayenne, chilli and paprika. Corn, squash, beans of all variety, papaya, avocado, pineapple and peanuts are indigenous in America. Maple syrup was an American Indian staple sweetener. In search for causal allergens of disease, their possible covert sources must be remembered. Corn oil is used in mayonnaise, oleomargarine, salad dressing, canning sardines, for deep frying doughnuts, potato chips, potatoes, fish, for brushing baked goods, in processing frozen fish, fruit and berries. Corn derivatives are utilized in bread, crackers, puddings, cakes, pies, breakfast foods, hot dogs, sausages, luncheon loafs, canned corned beef hash, canned and frozen foods, catsup, chili sauce, baby food, pickles, vinegar, preserves, marmalades, jams, jellies, fruit butters, fruit juices, ice cream and sherbet, beer, ale and soft drinks. Exposure to soy bean may occur through its addition to flour, breads, crackers, cereals, cakes, flakes, mayonnaise, salad oil, cooking oils, shortening, flavorings, chocolate coating, candies, and through its use in brewing and making wines. Hidden allergens in the form of additives, such as coloring, flavoring, preservatives, sweeteners, texturizers, stabilizers, constitute a true challenge to the diagnostic ingenuity of the clinician. The potential allergen tartrazine, an azobenzene dye, is often used in gelatine desert, ice cream and in foods of the same category. Seven out of eight aspirin-sensitive patients of Juhlin developed asthma, urticaria or both after taking 1 to 2 mg of tartrazine (J Allergy Clin Immunol 50:92, 1972). Other additives, sodium nitrate and sodium nitrite may be used in chopped, pickled, cured meats, ham and fish. In 1968, there were 2,700 different food additives available according to the National Research Council, quoted by Feingold, B F (Ann Allergy 26:309, 1968). Also, he lists items to which salicylates are added: ice cream, bakery goods (except bread), candy, chewing gum, soft drinks, jello, jams, cake mixes, wintergreen flavors. Inhalant allergens may originate from insects. It is less well known that in some apparently food-sensitive persons the respective food does not cause hypersensitivity symptoms but the cause of the latter is found in contamination of the food with insects or insect debris, as it may occur with the ingestion of cereals, corn meal, oat meal, grains, products made with flour, tomatoes, catsup, canned tomato juice, pickles, canned fruits, nuts, chocolate, and wines. The allergic pathogenicity of house dust has been clarified since the role of mites became known. Brown et al reported (Brit Med J 3:646, 1968) that in persons known or suspected of being allergic to house dust, *l. subtilis*, used in laundry products, have been identified 98.1 percent. Proteolytic enzymes derived from *Bacillus* positive skin test to four different mites varied from 35.6 per cent as causal agents of allergic rhinitis, nasal stenosis, sneezing, itching of the hands and of the eye lids, lacrimation, redness of the eyes, cough, chest tightness and wheezing. No doubt a great many nonsmokers, particularly children, are exposed to second-hand cigarette smoke in their homes. Clinical investigations have ascertained that exposure of this type may exert adverse effect upon the respiratory tract and also result in eye irritation and migraine. Aggravation of bronchial asthma or asthmatic children was recorded by O’Connell et al (Annual Meet AMA, 1971) in 20 percent of these nonsmoking patients from homes where both parents were smoking and in 10 percent of homes where one parent smoked. Even though this writing offers only partial coverage of the subject, it seems justifiable to say that the magnitude and complexity of health problems resulting from nutritional and ecologic influences of the home call for urgent commensurate attention to them.

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