Idiopathic Pulmonary Hemosiderosis: An Electron Microscopic and Immunofluorescent Study*

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Electron microscopic and immunofluorescent studies are reported in a patient with idiopathic pulmonary hemosiderosis, suggesting that idiopathic pulmonary hemosiderosis is distinct from other pulmonary hemorrhagic syndromes associated with antibasement membrane antibody or immune complex mediated diseases. The ultrastructural studies showed evidence of nonspecific lung injury and indirect evidence of vascular lesions. No subendothelial deposits or basement membrane lesions were seen. Immunofluorescent studies failed to reveal any evidence for localization of IgG, IgM, ß2C, Clq or fibrinogen in the lung. Investigation of platelet function showed low and low normal values of platelet retention on a glass bead column, but findings were otherwise normal.

Idiopathic pulmonary hemosiderosis (IPH) is a disorder of unknown etiology and unclear pathogenesis characterized by recurrent episodes of intrapulmonary hemorrhage. Clinically, the triad of hemoptysis, diffuse parenchymal infiltrates on chest roentgenogram, and iron deficiency anemia is sufficiently common in patients with IPH to suggest the diagnosis;¹ but it is not specific since other diseases can present in the same manner.²³ Pathologically, degeneration, shedding and hyperplasia of alveolar epithelial cells have been described as the "basic" microscopic lesion of IPH.⁴ However, these changes can be caused by a variety of agents⁵ and are a reflection of nonspecific lung injury. Since the clinical features and light microscopic findings can all be caused by chronic, recurrent bleeding episodes from any cause, and since no criteria have been established or followed in order to make the diagnosis of IPH, skepticism and confusion should exist regarding IPH as a distinct entity. The purposes of this paper are to present electron microscopic and the first immunofluorescent studies on lung tissue from a patient with IPH, with emphasis on the necessity of extensive investigation in order to exclude other diagnostic possibilities.

Case Report

A 21-year-old Cuban-born man was admitted on Nov. 8, 1971 with the chief complaint of coughing up blood-streaked sputum for two months.

Two years previously he had been admitted for evaluation of iron deficiency anemia, with a hematocrit value of 27 percent. Chest roentgenogram and extensive evaluation of the gastrointestinal (GI) tract showed normal findings. He was discharged with the diagnosis of iron deficiency anemia of unknown etiology and treated with orally administered iron therapy for six months. For the next year and one half the patient was asymptomatic and had a normal hematocrit level.

Two months prior to admission, the patient noted fatigue and a constant cough, with blood-streaked sputum. These symptoms persisted and he was readmitted. At this time the pertinent findings included physical examination, which showed no abnormalities; a chest roentgenogram showing diffuse hazy densities in both lower lobes; an arterial oxygen pressure (Pao₂) of 75 torr on room air; and iron deficiency anemia, with a hematocrit level of 27 percent; a serum iron value of 30 μg/deciliter and a total iron binding capacity of 505 μg/deciliter. Over the ensuing few days, the cough became intermittent, with continuing occasional hemoptysis of small amounts of blood, the alveolar infiltrates on the chest roentgenograms resolved, leaving minimal bilateral reticulonodular densities, and the Pao₂ reading returned to normal levels. Other laboratory abnormalities included a positive test

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result from purified protein derivative (PPD), first noted two years previously, eosinophilia (180 to 900/mm³), and type B Wolf-Parkinson-White pattern on ECG.

In order to investigate the multiple causes of hemoptysis in our patient, the following evaluation of his cardiorespiratory, renal, clotting, immunologic and genetic status was performed. Results of pulmonary function tests including diffusing capacity, bilateral bronchograms, sinus films and ultrasonography of the mitral valve were all normal. Sputum cultures grew normal flora but no fungi or tubercle bacilli. Findings from five urinalyses, creatinine clearance and 24-hour urine protein were within normal limits. Partial thromboplastin time, prothrombin time, fibrinogen, euglobulin lysis time, bleeding time, platelet factor III, platelet count and clot retraction were all within normal limits. Platelet retention on a glass bead column on two occasions was low (11 percent) and low normal (28 percent), the normal value being greater than 25 percent. The patient had not been taking any medication for two weeks when the coagulation studies were performed. Serum protein electrophoresis, serum immunoelectrophoresis, serum complement in 50 percent units were all normal. ANA, RF, mycoplasma titers, cold agglutinins, cryoglobulins, and lupus erythematosus (LE) preparations revealed no abnormalities. Serum antibodies to lung and kidney basement membrane were not detected by the indirect, double-layer immunofluorescence technique, using sections of human lung and kidney. Serum from a patient with Goodpasture’s syndrome was used as a positive control. Results of karyotype analysis, using a Giemsa-banding technique, were normal.

Following this evaluation, an open lung biopsy was performed through a limited left thoracotomy on Nov. 30, 1971 and showed pulmonary hemosiderosis. Subsequently, the patient was discharged on iron therapy orally, with the diagnosis of IPH. Nineteen months following discharge, the patient complains of an occasional cough, with intermittent blood-streaked sputum. His hematocrit, urinalysis and sputum cultures grew normal flora but no fungi or tubercle bacilli. Subsequently, the patient was discharged on iron therapy orally, with the diagnosis of IPH. Nineteen months following discharge, the patient complains of an occasional cough, with intermittent blood-streaked sputum. His hematocrit, urinalysis and serum complement values are normal.

**Materials and Methods**

At thoracotomy, samples of obviously abnormal and apparently normal lung tissue were obtained.

Tissue for light microscopic study was fixed in Bouin’s solution and studied with the following stains: hematoxylin, phloxin and saffron; Perl’s iron; Masson’s trichrome; Verhoeff’s elastic; and Laidlaw’s reticulin.

Tissue for immunofluorescent study was snap frozen in alcohol and dry ice. Sections of this tissue, four microns in thickness, were stained with fluorescein conjugated antisera to human IgG, IgM, βC, Clq and fibrinogen. The method of antisera preparation, conjugation and staining has been described previously.

Tissue for electron microscopic study was fixed overnight in buffered 2.5 percent glutaraldehyde solution at room temperature and at a pH of 7.6. Then it was rinsed in buffer and fixed again in buffered osmium tetroxide for one hour at room temperature. The blocks were next dehydrated in graded acetone solutions and embedded in Durcupan (Fluka AG Chemische Fabrik, Buchs SG, Switzerland). Thin sections were mounted on Formvar-coated copper grids, stained with lead citrate and uranyl acetate and examined with a Siemens Elmskop 101 electron microscope.

**Results**

Sections of apparently normal and obviously ab-

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**IMMUNOFLOUORESCENCE**

Immunofluorescent studies showed no localization of IgG, IgM, β1C, Clq or fibrinogen in the lung.

**ELECTRON MICROSCOPY**

The alveolar spaces contained red blood cells, edema fluid, fibrin and macrophages filled with dense pleomorphic granules having the ultrastructural characteristics of hemosiderin12 (Fig 1, 2, 3). No abnormality of either alveolar capillary endothelium or basement membranes could be found (Fig. 4). Moreover, red blood cells were also seen enmeshed in bands of collagen fibers in the interstitium of interlobular septa, with no capillaries or alveoli in proximity (Fig 5). Even though the alveolar lining appeared normal in most regions, small portions of exposed basement membrane from degeneration of type I pneumocytes were consistently found (Fig 6). Also, type II pneumocytes were increased in number and at times found side by side (Fig 7).

**DISCUSSION**

The clinical and pathologic manifestations of IPH1 are nonspecific because they can be caused by a variety of conditions in which bleeding into the

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*Figure 3. Electron micrograph showing part of edema-filled alveolus (A), fresh erythrocyte (RBC) in interstitium surrounded by collagen fibrils (C) and elastic tissue (El). Epithelium (I) and its basement membrane (*) appear normal. (x 7,500; lead citrate and uranyl acetate stains).*

*Figure 4. Electron micrograph representative of most of tissue examined. Erythrocytes (RBC) are seen in alveolar space (A) and in alveolar capillaries (C). Type II pneumocyte (II) is present in upper part of field. Nucleus of type I pneumocyte (I) is seen. Normally thin type I cell processes (†) extend over alveolar surface. Nucleus of capillary endothelial cell (E) is present. Its cell processes line capillary lumen. Alveolar-capillary basement membrane is normal, being uniform in width and density (x 12,000; lead citrate and uranyl acetate stains).*
Electron micrograph showing portion of interlobular septum with fresh hemorrhage in form of intact erythrocytes (RBC) enmeshed in bands of collagen fibers (CT) of connective tissue stroma (x 2,250; lead citrate and uranyl acetate stains).

Figure 5. Electron micrograph showing portion of interlobular septum with fresh hemorrhage in form of intact erythrocytes (RBC) enmeshed in bands of collagen fibers (CT) of connective tissue stroma (x 2,250; lead citrate and uranyl acetate stains).

l lung occurs. Hemosiderosis of the lung has occurred in association with intrapulmonary hemorrhage in the following conditions: mitral stenosis or other conditions with chronic venous hypertension,2 periarteritis nodosa,3,4 Wegener's granulomatosis,5 systemic lupus erythematosus,6 Goodpasture's syndrome or antiglomerular basement membrane antibody mediated disease,7 and viral pneumonitis.8 In fact, the mere introduction of blood into the lung appears to cause a morphologic picture indistinguishable from IPH. Both intratracheal instillation of blood in experimental animals,14 and bleeding from a localized carcinoma into adjacent lung segments in humans beings8 have led to degeneration, shedding and hyperplasia of alveolar cells, associated with blood, hemosiderin and hemosiderin laden macrophages in the alveoli. Other clinical features of the above mentioned diseases which can also mimic IPH are the alveolar roentgenographic pattern seen after any pulmonary bleeding, and the iron deficiency anemia caused by pulmonary deposition of iron. Consequently, since the clinical course and pathologic findings of IPH can be caused by a variety of other diseases, the diagnosis of IPH must be one of exclusion.

Despite the usefulness of light microscopy in making the diagnosis of these other conditions, some disease entities may continue to masquerade as IPH unless electron microscopic and immunofluorescent investigations are also carried out. A patient reported as having IPH illustrates this concept.15 Even though the clinical course and light microscopic appearance of his lung tissue were compatible with IPH, electron microscopy revealed probable subendothelial immune complexes. This patient subsequently has developed systemic lupus erythematosus,6 an immune complex mediated disease, demonstrating that electron microscopy may suggest the diagnosis when other investigations have failed.

Electron microscopic and immunofluorescent studies were performed on our patient's lung tissue. Three findings of the electron microscopic studies merit discussion. First, the finding of red blood cells in the alveolar interstitium and the connective tissue stroma of the interlobular septa is evidence that the vascular abnormality is widespread and not limited to alveolar capillaries. Second, basement membranes were normal, and despite extensive search, no specific vascular abnormalities to account for the diffuse bleeding were found. Hyatt et al15 on the other hand, found evidence of localized alveolar capillary damage in a specimen obtained by needle lung biopsy. They found a single break in the capillary

Figure 6. Electron micrograph showing capillary and part of alveolus, with fragmentation of type I alveolar lining cell (tf). Basement membrane (*), endothelial cells (On), and cell junctions (J) appear normal. Granular material associated with edema fluid is present in alveolus (A), and clear spaces indicating interstitial edema (ES) are present in connective tissue adjacent to capillary (x 7,500; lead citrate and uranyl acetate stains).

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Figure 7. Electron micrograph showing two adjacent type II epithelial cells (II) on alveolar septum, suggesting previous epithelial damage. Interstitial edema (ES) is present in septum. All other structures appear normal. Erythrocytes (RBC) are seen in alveolus at bottom of micrograph (x 1,950; lead citrate and uranyl acetate stains).
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basement membrane with an adjacent degenerated endothelial cell and an adjacent distorted type I pneumocyte. Our failure to find such a lesion is not necessarily contradictory, since only small amounts of tissue are sampled with the electron microscope and it is conceivable that the lesion is transient or only detectable early in an episode of pulmonary hemorrhage. Third, the findings of degeneration of type I pneumocytes and increased numbers of type II pneumocytes are both nonspecific: the former occurs in many types of lung damage,18 and the latter is thought to represent a stage of lung repair.19 Thus, it is impossible to say whether these last changes were related to the cause of the bleeding or were merely part of the lung’s reaction to blood in the alveoli.

The findings of the immunofluorescent studies were all negative. There was no detectable antibody to lung or kidney basement membrane in our patient’s serum and no localization of IgG, IgM, β2C, Clq or fibrinogen in his lung. Although immunofluorescent studies on lung tissue from patients with IPH have never been reported before, it has been proposed that IPH may be an immunologically mediated disease,19 and more specifically a variant of Goodpasture’s syndrome.20,31 We found no evidence to support these hypotheses.

Since the mechanism of bleeding could conceivably be related to a hemorrhagic diathesis, we also performed clotting studies including extensive tests of platelet function not reported before in patients with IPH. All results were within normal limits except for low and low normal values of platelet retention on a glass bead column. The significance of this isolated finding can only be answered by further studies.

Despite this detailed investigation, two basic questions concerning IPH remain. First, does IPH exist distinct from other classified pulmonary diseases? Although this question cannot be unequivocally answered at present, our case and that of Hyatt et al18 suggest that IPH exists as a distinct entity because both cases showed pulmonary hemosiderosis and failed to show evidence of inflammation, vasculitis, necrosis, immune complexes, or gamma globulin and complement directed against basement membrane. Second, what is the site and nature of the lesion that causes the bleeding? Our finding of red blood cells in alveolar and nonalveolar interstitial regions is strong evidence for a diffuse vascular abnormality in the lungs of patients with IPH. Although the break in the capillary membrane demonstrated by Hyatt et al18 may well be an example of the specific abnormality, further confirmation is necessary.

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