Idiopathic pulmonary fibrosis associated with high-titer antibodies against nuclear ribonucleoprotein (nRNP): Report of 3 cases

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Three patients with idiopathic pulmonary fibrosis (IPF) who were found to have high-titer antibodies directed against nuclear ribonucleoprotein are reported. All patients had nonspecific pulmonary symptoms, increased interstitial markings on chest radiographs, restrictive pulmonary function test results, elevated levels of circulating immune complexes, a histologic pattern consistent with IPF, and, when studied, positive immunofluorescence in the lung tissue for IgM, IgG, and the third component of complement. HLA typing revealed 2 patients to have the DR1 phenotype and all 3 to have the MT1 phenotype, although the patients were of different racial backgrounds. Although extensive evaluation failed to reveal evidence of an underlying collagen vascular disease, these patients were serologically, radiographically, histologically, and genetically similar to patients with the IPF that is associated with mixed connective tissue disease (MCTD). The authors suggest that these patients may be exhibiting an incomplete form of MCTD and that these 3 cases lend further support to an interrelationship between IPF and the collagen vascular diseases.

Idiopathic pulmonary fibrosis (IPF) without an associated collagen vascular disease was first described by Hamman and Rich[1] in 1933. Although a great deal has been reported about the clinical course of this disease, the etiology and pathogenesis remain obscure.[2]

Over the past decade, several studies have produced evidence that idiopathic pulmonary fibrosis may represent the pulmonary manifestation of an immunologically mediated systemic disease. Indeed, it has been increasingly recognized that the lung disease of IPF closely resembles both clinically and histologically the lung disease associated with the autoimmune collagen vascular diseases. However, the exact immunologic mechanisms involved in the pathogenesis of IPF are still undetermined. Several studies have reported that rheumatoid factors,[2-4] antinuclear antibodies,[2-5] and circulating immune complexes[6] are frequently present in the sera of patients with IPF—with or without an associated autoimmune disease. Furthermore, immunohistochemical techniques have demonstrated the deposition of immune complexes and complement in the lungs of patients with active interstitial disease.[6] Such similarities suggest that some patients who have IPF without an associated disease may represent a forme fruste of a collagen vascular disease.

In recent years, immunologic techniques that can determine the antigenic specificity for various autoantibodies have been developed. Frequently, the antibody is found to be directed against a nuclear antigen that is highly suggestive of a particular disease.[7-13] Although immunologic analysis of these antinuclear antibodies (ANAs) and their significance has been determined for the defined collagen vascular diseases, the ANAs that are present in IPF without a defined disease has rarely been similarly investigated.[14] Thus, we wish to report 3 patients who presented with IPF and a positive antinuclear antibody but who did not have other features of an associated collagen vascular disease. On further immunologic analysis, these patients' ANAs were found to be directed against the antigen ribonucleoprotein (nRNP). Identification of these 3 patients helps to further support an interrelationship between IPF and the collagen vascular diseases.

Patients and methods

Patient identification

Three patients who were treated at Fitzsimons Army Medical Center for histologically proven idiopathic pulmonary fibrosis were studied extensively. Each patient was chosen for further evaluation on the basis of a positive antinuclear antibody test, with the ANA directed against the specific antigen nRNP. None of the patients fulfilled any American Rheumatism Association criteria[15] for one of the collagen vascular diseases.

Serologic studies

Blood was obtained from each patient by venipuncture and was immediately processed for analysis. Each patient's sera were analyzed for the presence of antinuclear antibody, rheumatoid factor, total hemolytic complement, and circulating immune complexes. Each patient's lymphocytes were sent for human leukocyte antigen (HLA) typing.

Antinuclear antibodies were determined by indirect immunofluorescence using mouse kidney sections and Hep-2 cells as substrates. Sera positive by indirect immunofluorescence were then analyzed to determine whether the autoantibodies were directed against specific nuclear antigens. Antibodies to double-stranded DNA were tested for by radioimmunoassay using the Millipore filter technique.[16] Antibodies to rheumatoid arthritis nuclear antigen and SS-A antigens were tested for by Ouchterlony immunodiffusion using prototypic antisera with known activity against one antigen and Wil_2 cell extracts as the antigen source.[7,17] Antibodies to SS-B, Sm, RNP, and Scl-70 antigens were tested for by Ouchterlony immunodiffusion using rabbit thymus extract as the antigen source.[10] Titers were determined for positive results by counterimmunoelectrophoresis (CIE) and passive hemagglutination techniques using prototypic antisera with known reactivity against these antigens.[8,17,18] Antibodies to centromere were determined using an indirect immunofluorescence technique with Hep-2 cells as substrate for analysis of specific metaphase immunofluorescent patterns.[9] All tests were performed in duplicate by the Rheumatology Laboratory at the University of Colorado Health Sciences Center.

We determined rheumatoid factor by latex agglutination,[19] total hemolytic complement by lysis of sheep red blood cells,[20] and circulating immune complexes by C1Q binding assay.[21]

HLA typing using the standard National Institutes of Health lymphocyte microcytotoxicity assay was performed by an experienced technician using a minimum of three typing antisera for one antigenic specificity.[22] The typing sera were a combination of the third American histocompatibility workshop and local typing sera obtained from the National Institutes of Health. All tests were performed in duplicate by the Tissue Transplantation Laboratory at Walter Reed Army Medical Center.

Immunofluorescence

Goat antisera to human IgG, IgM, IgA, the third component of complement (C3), fibrinogen, and albumin conjugated with fluorescein isothiocyanate were obtained from Calbiochem-Behring Corporation, La Jolla, California.

Lung biopsy specimens were covered with Ames imbedding medium and snap frozen in dry ice; 4-micron sections were air dried for 10 minutes and then fixed in acetone for an additional 5 minutes. Each section then had three successive, 5-minute washes of phosphate-buffered saline solution. The sections were dried, and, after 30 minutes of exposure to conjugated antisera at room temperature, they were again washed with phosphate-buffered saline during 3 successive, 5-minute periods. The sections were then coverslipped and examined under a fluorescent microscope. A lupus kidney section that was known to contain IgG, IgM, and C3 served as a positive control.

Report of cases

Case 1

A 46-year-old American Indian woman was admitted in April 1982 for evaluation of increasing shortness of breath and an abnormal chest x-ray. History review showed that the patient had been in good health until 1976. At that time, she had begun to experience recurrent episodes of "pneumonia." Over the next several years, she had developed a chronic nonproductive cough, increasing shortness of breath, and dyspnea on minimal exertion. She had a 20 pack-year history of smoking, but she had voluntarily stopped smoking in 1980. In 1981, after a "pneumonia" episode, she had been treated empirically for 1 year with isoniazid and rifampin for suspected tuberculosis, although all cultures had been negative. She failed to improve on the antituberculous therapy. She denied exposure to environmental pneumotoxins and all symptoms suggestive of collagen vascular disease.

On admission, physical examination revealed a slightly obese woman who was in mild respiratory distress. Pulmonary examination revealed dry inspiratory rales without rhonchi or wheezes. Cardiac examination showed no accentuation of the pulmonic component of the second heart sound. The patient had mild clubbing of the fingers and toes, but no cyanosis or edema. The remainder of

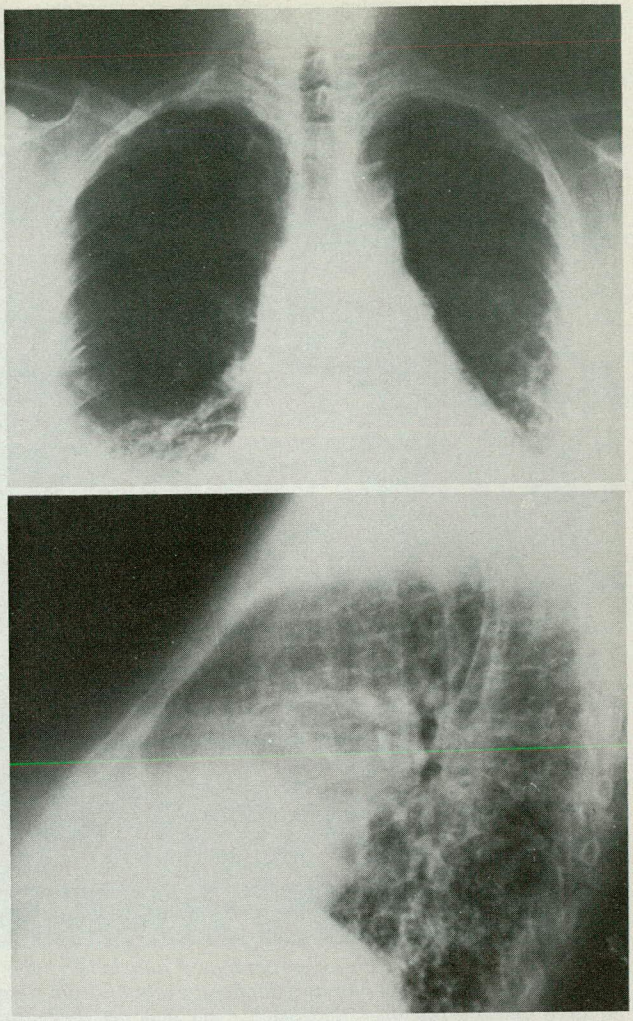
the physical examination was unremarkable.

Laboratory examination revealed normal cell count, blood chemistry values, serum creatine phosphokinase level, urinalysis results, and coagulation times. The Wintrobe sedimentation rate was elevated to 45 mm./hr. Serologically, tests for rheumatoid factor, total hemolytic complement, quantitative immunoglobulin, cryoglobulins, serum protein electrophoresis, rapid plasma reagin (RPR) test, and Coombs' test had either normal or negative results. An ANA test was positive at a titer ≥1:256 in a speckled pattern. Analysis of the ANA revealed it to be of the IgG class and directed against nRNP titers of 1:16 by CIE and 1:5,000 by passive hemagglutination. Testing for antibodies to rheumatoid arthritis nuclear antigen, SS-A, SS-B, Sm, Scl-70, and centromere were all negative. Circulating immune complex levels were elevated. HLA typing revealed the following phenotype: HLA A2, 24; B35, 40; CW4; DR6, 9; MT1.

Pulmonary evaluation included a chest x-ray, which demonstrated interstitial markings in the lower lobes more than the upper lobes (Figs. 1A and 1B). Pulmonary function test results were consistent with restrictive lung disease: forced vital capacity (FVC), 2.08 L./sec. (2.94 L./sec. predicted value); forced expiratory volume in 1 second (FEV_1), 2.03 L./sec., (2.39 L./sec. predicted value); FEV_1:FVC ratio, 97.7 percent; total lung capacity (TLC), 3.25 L. (4.18 L. predicted value); residual volume (RV), 1.14 L. (1.39 L. predicted value); and diffusing capacity for carbon monoxide (D_LCO) 40 percent of predicted value. An exercise study was significant for an 11 percent drop in oxygen saturation. Arterial blood gas determinations revealed a pH of 7.43, an oxygen partial pressure (PO_2) of 56 mm. Hg, and a carbon dioxide partial pressure (PCO_2) of 38 mm. Hg. Other diagnostic studies included normal lip biopsy showing no evidence of Sjögren's syndrome, normal barium swallow showing no evidence of dysmotility, and negative gallium scan of the lungs.

In June 1982, the patient underwent open lung biopsy. The histologic pattern was consistent with idiopathic pulmonary fibrosis with mild residual chronic inflammation (Figs. 2A and 2B). Unfortunately, no tissue immunofluorescence studies were performed. There was no evidence of granuloma, vasculitis, or obliteration of the vasculature. All bacterial, fungal, and mycobacterial cultures were negative. Bronchial lavage was not performed.

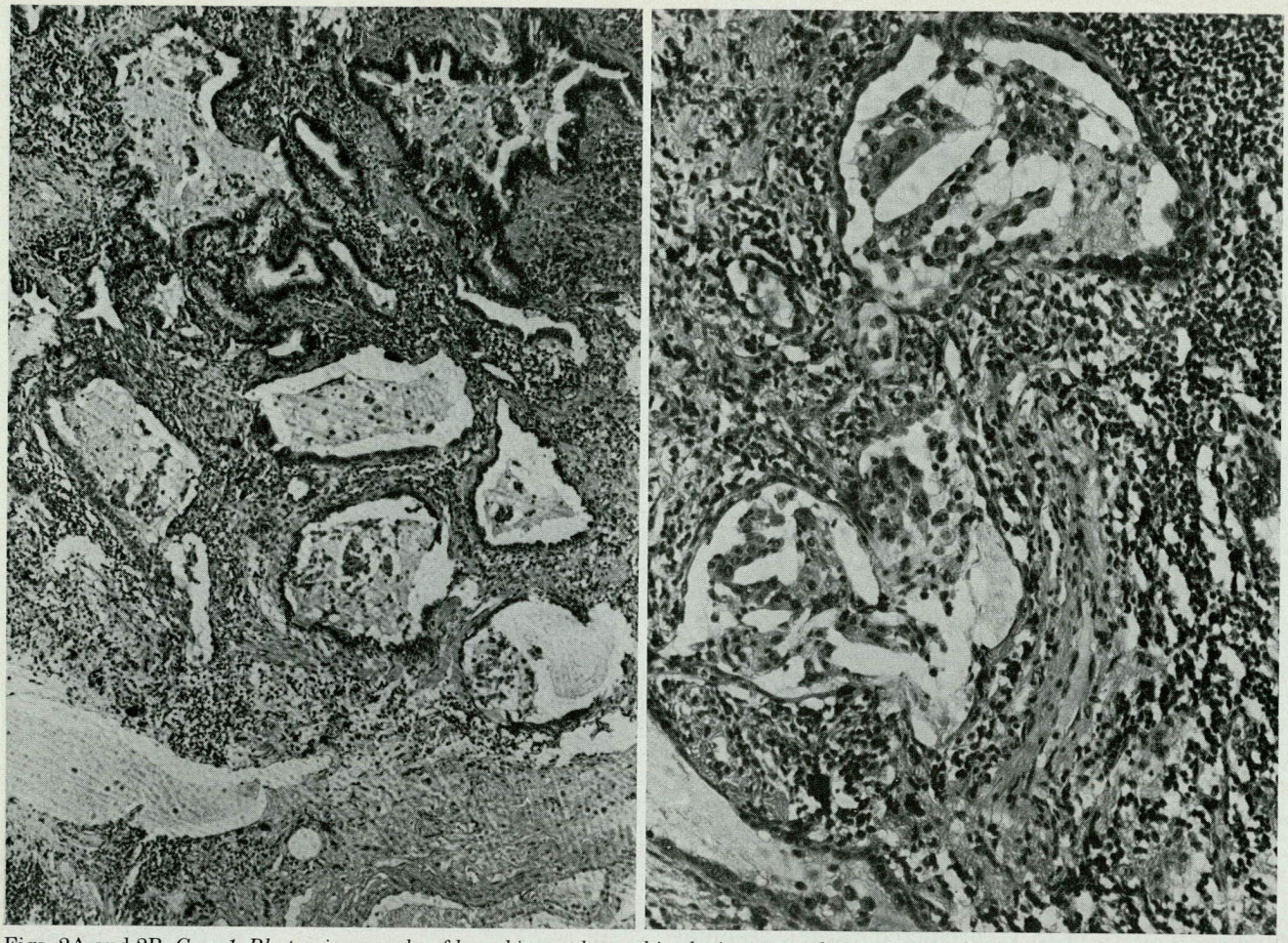
Postoperatively, the patient was placed on high-dose prednisone (100 mg. per day in divided doses). This dosage was lowered to 60 mg. per day over the next month. After 3 months on this regimen there was little change in the patient's symptomatology or pulmonary functions. Her prednisone was tapered to 10 mg. a day, which she remains on without further deterioration of her pulmonary status.



Figs. 1A and 1B. *Case 1. Posteroanterior (A) and lateral (B) chest radiographic views demonstrate predominant lower lobe interstitial pattern.*

Case 2

A 34-year-old black woman was admitted in April 1978 for evaluation of increasing shortness of breath. History review revealed that the patient was first evaluated in 1976, when a routine chest x-ray for a physical examination had exhibited increased interstitial markings. Evaluation at that time had revealed normal arterial blood gas values, but pulmonary function tests had shown a mild restrictive pattern. Because of the patient's asymptomatic state, it had been elected not to treat her at that time. She had been lost to follow up until 1978, when she returned with a nonproductive



Figs. 2A and 2B. *Case 1. Photomicrographs of lung biopsy show a histologic pattern that is consistent with IPF.* Fig. 2A *demonstrates severe interstitial fibrosis, chronic lymphocytic cell infiltrate, desquamative type of pattern in several terminal airways with eosinophilic material and macrophages, and reactive pneumocytes (hematoxylin and eosin stain; original magnification, ×100).* Fig. 2B *exhibits terminal airways with desquamative and inflammatory process (hematoxylin and eosin stain; original magnification, ×250).*

cough, increasing shortness of breath, and dyspnea on exertion. Review of systems was remarkable for a 3 pack-year history of smoking. She denied exposure to environmental pneumotoxins and all symptoms suggestive of a collagen vascular disease.

On admission, physical examination revealed a thin woman in mild respiratory distress. Pulmonary examination demonstrated dry inspiratory rales without rhonchi or wheezes. Cardiac examination showed no accentuation of the pulmonary component of the second heart sound. The patient had no clubbing, cyanosis, or edema. The remainder of the physical examination was unremarkable.

Laboratory studies revealed a normal cell count, blood chemistry levels, coagulation times, serum creatine phosphokinase value, and urinalysis results. The Wintrobe sedimentation rate was elevated to 33 mm./hr. Serologic examinations showed the rheumatoid factor, total hemolytic complement, RPR, Coombs', and cryoglobulin test results to be normal or negative. Serum protein electrophoresis showed polyclonal gammopathy secondary to elevation of the IgG component. An ANA test was positive at a titer $\geq$1:256 in a speckled pattern. Analysis revealed the ANA to be of the IgG class and directed against nRNP at titers of 1:32 by CIE and 1:20,000 by passive hemagglutination. Studies for antibodies to rheumatoid arthritis nuclear antigen, SS-A, SS-B, Sm, Scl-70, and centromere were all negative. Circulating immune complex levels were elevated. HLA typing showed the following phenotype: HLA A1, 28; B7, 12; CW2, 4; DR1, 4; MT1.

On pulmonary evaluation, chest roentgenography showed increased interstitial markings, more in the lower lobes than in the upper lobes. Pulmonary function study findings were consistent with a restrictive disease: FVC, 2.19 L./sec. (3.29 L./sec. predicted value); FEV_1, 2.16 L./sec. (2.27 L./sec. predicted value); FEV_1:FVC ratio, 98 percent; TLC, 3.09 L. (4.60 L. predicted value); RV, .9 L. (1.54 L. predicted value); and D_LCO, 32 percent of predicted value. An exercise study was significant for a 12

percent drop in oxygen saturation. Arterial blood gas determinations revealed the following values: pH, 7.46; PO_2, 64 mm. Hg; and PCO_2, 33 mm. Hg. Other diagnostic studies included normal Schirmer's and rose bengal tests, normal barium swallow showing no evidence of dysmotility, and negative gallium scan of the lungs.

In 1979, the patient underwent open lung biopsy. Histologically, the findings were consistent with idiopathic pulmonary fibrosis with moderate residual chronic inflammation. Immunohistochemical staining of lung tissue revealing deposition of IgM, IgG, and the third component of complement. The staining was most marked in the interstitial spaces in the areas of active inflammation. There was no evidence of granulomas, vasculitis, or obliteration of the vasculature. Bacterial, fungal, and mycobacterial culturing had negative results. Bronchial lavage was not performed.

Following biopsy, the patient was placed on high-dose prednisone (60 mg. per day in divided doses), which was continued for 3 months without significant improvement. Consequently, she was tapered to 15 mg. per day over the next 3 months, and she remained on that regimen for an additional 6 months. Pulmonary function tests in 1981 showed some mild worsening. No further data are available because the patient has been lost to follow up since early 1982.

Case 3

A 19-year-old white man was admitted for evaluation of increasing dyspnea on exertion. His past history showed that the patient had been in good health until 1975, when he had presented to the pediatric clinic complaining of some mild shortness of breath and inability to keep up with his peers in sports. Evaluation at that time had revealed a normal chest x-ray; however, pulmonary function tests had shown a mild restrictive pattern. For unclear reasons, no further evaluation or treatment was given. The patient had had no further follow up until October 1981. Review of systems was remarkable for a chronic, nonproductive cough and some mild dyspnea on exertion. The patient also reported some mild cold sensitivity of his hands (not true Raynaud's phenomenon). He denied exposure to environmental pneumotoxins and all other symptoms suggestive of collagen vascular disease. The patient did not smoke.

Physical examination on admission revealed a thin man in no respiratory distress. Pulmonary examination demonstrated dry inspiratory rales without rhonchi or wheezes. Cardiac examination showed no accentuation of the pulmonic component of the second heart sound. The patient had no evidence of clubbing, cyanosis, or edema. The remainder of the physical examination was unremarkable.

Laboratory examination revealed normal cell count, blood chemistry, serum creatine phosphokinase, urinalysis, and coagulation studies. The Wintrobe sedimentation rate was elevated to 43 mm./hr. Serologically, rheumatoid factor, total hemolytic complement, RPR, Coombs', and cryoglobulin study findings were all normal or negative. Serum protein electrophoresis revealed a mild polyclonal gammopathy secondary to an elevation of the IgG component. ANA testing was positive at a titer $\geq$1:256 in a speckled pattern. Analysis revealed the ANA to be of the IgG class and directed against nRNP at titers of 1:64 by CIE and 1:160,000 by passive hemagglutination. Testing for antibodies to rheumatoid arthritis nuclear antigen, SS-A, SS-B, Sm, Scl-70, and centromere were all negative. Circulating immune complex levels were elevated. HLA typing revealed the following phenotype: HLA A2, 26; B5, 15; CW1, 4; DR1, 5; MT1.

Pulmonary evaluation included a chest x-ray, which showed increased interstitial markings in the lower lobes more than the upper lobes. Pulmonary function test results were consistent with a restrictive disease: FVC, 1.98 L./sec. (3.51 L./sec. predicted value); FEV_1, 1.87 L./sec. (3.01 L./sec. predicted value); FEV_1:FVC ratio, 92 percent; TLC, 3.54 L. (4.7 L. predicted value); RV, 1.1 L. (1.7 L. predicted value); and D_LCO, 50 percent of predicted value. An exercise study was significant for a 13 percent drop in oxygen saturation. Arterial blood gas determinations revealed a pH of 7.46, PO_2 of 73 mm. Hg, and PCO_2 of 35 mm. Hg. Other diagnostic studies included normal Schirmer's and rose bengal tests, normal barium swallow showing no evidence of dysmotility, and negative gallium scan of the lungs.

In June 1982, the patient was taken to open lung biopsy. The histologic evaluation was consistent with interstitial pulmonary fibrosis with moderate residual chronic inflammation. Immunohistochemical staining of the lung tissues was positive for IgM, IgG, and the third component of complement being deposited in areas of maximum inflammation. There was no evidence of granuloma, vasculitis, or obliteration of the vasculature. All bacterial, fungal, and mycobacterial cultures were negative. Bronchial lavage was not performed.

Postoperatively, the patient was placed on a regimen of 50 mg. of prednisone once a day. This was continued for the next 3 months without a significant change in his symptomatology or pulmonary

TABLE 1. AUTOANTIBODIES PRESENT IN THE COLLAGEN VASCULAR DISEASES.

Disease	Autoantibody
Mixed connective tissue disease[10]	Anti-nRNP (high titer)
Progressive systemic sclerosis[8,9]	Anti-Scl-70
CREST syndrome[9]	Anti-centromere
Systemic lupus erythematosus[12]	Anti-ds DNA, anti-Sm
Sjögren's syndrome[7]	Anti-SS-B
Polymyositis[11,13]	Anti-PM-1
	Anti-Jo-1

functions. Consequently, the patient's steroids were slowly decreased to 20 mg. per day over the next year, with a mild deterioration being observed on pulmonary function testing. At that time he was placed on Imuran (100 mg. per day), which appeared to stop progression of his lung disease over the next 6 months. The patient has remained in stable condition on low-dose prednisone and Imuran until his transfer in 1983.

Comment

In 1976, Crystal and associates[2] summarized the currently accepted classification and diagnostic criteria for idiopathic pulmonary fibrosis. This classification proposes that there are two defined groups of patients with IPF: The first is composed of patients who have IPF that is associated with a defined collagen vascular disease; the second consist of patients who have no associated disease. However, despite this arbitrary division, many similarities have been demonstrated both serologically and histologically,[2-6,23-26] which suggests that the pathogenesis of the lung disease in these two groups of patients may overlap.

Several of the collagen vascular diseases have IPF as part of their disease spectrum. For unknown reasons, rheumatoid arthritis, mixed connective tissue disease, and scleroderma have been more commonly associated with IPF than have systemic lupus erythematosus, Sjogren's syndrome, and polymyositis.[26]

Serologically, each of the collagen vascular diseases frequently demonstrates the presence of antinuclear antibodies. In recent years, sophisticated immunologic techniques have been used to analyze these autoantibodies, and this evaluation has demonstrated that a certain percentage of ANAs are directed against nuclear antigens that are thought to be highly suggestive of each disease (Table 1).[7-13] The diagnostic importance of having a serologic marker for each disease is evident. However, their pathogenetic significance is being debated, with some investigators believing that ANAs only represent an epiphenomenon resulting from persistent immune stimulation, while other researchers think that ANAs may play a central role in the pathogenesis of collagen vascular diseases through the formation of circulating immune complexes that are deposited in various tissues and thus result in altered function.[28]

The etiology and pathogenesis of lung disease in patients with IPF without associated collagen vascular disease is under investigation. Previous reports[2-6,23,24] have stressed that these patients can have serologic markers and lung histology identical to that associated with the collagen vascular diseases. Indeed, rheumatoid factors and ANAs have been demonstrated in the sera of 7-49 percent[2-5] of patients with IPF not associated with a collagen vascular disease. Moreover, previous investigation has demonstrated the presence of immune complexes in the sera and lung tissue of many of these patients.[6] This suggests that a systemic immune-complex-mediated (type III) mechanism may have a pathogenetic role in some patients with IPF. These data further suggest that some cases of IPF alone, particularly when the disease is associated with a positive ANA, may have a close interrelationship with the collagen vascular diseases.

The 3 patients in this report have IPF and a positive ANA without other clinical evidence of a collagen vascular disease. On further analysis, the autoantibody in each case was found to be directed against the antigen, nRNP. Such antibodies have been reported to occur in several of the recognized collagen vascular diseases[10,12,29-40] and recently in cryptogenic fibrosing alveolitis (i.e., idiopathic pulmonary fibrosis).[14] However, extremely high titers of antibodies to nRNP without the simultaneous occurrence of antibodies to other nuclear antigens is highly characteristic of patients with mixed connective tissue disease (MCTD).[32, 41] Further analysis of these anti-RNP antibodies in patients with MCTD show them to be predominantly of the IgG class[32] and to be directed against an antigenic determinant that is located on a small nuclear RNA-protein complex, designated U1, which is involved in splicing, a reaction that is an integral part of mRNA processing.[42] One recent study[43] reported that antibodies to nRNP may penetrate live mononuclear cells through Fc receptors. Perhaps by complexing with the nuclear antigenic determinants, the function of such cells are altered, thus resulting in the autoimmune state.

Several indirect lines of evidence support an interrelationship between the lung disease exhibited by our patients and the lung disease that can affect up to 80 percent of patients with MCTD.[35,44-50] Indeed, both groups of patients serologically dem-

onstrated the presence of a specific autoantibody of the IgG class that was directed against nRNP. Furthermore, immune complexes have been demonstrated in the sera[51] and lung tissue[48] of both patient groups. Although not proven, it is intriguing to speculate that some of the anti-RNP antibodies complexed with their specific nuclear antigen may account for part of the circulating immune complexes that are deposited in the lungs.

Finally, although our number of patients is small, HLA typing demonstrated that these individuals of different racial backgrounds have the HLA DR1 and/or MT1 phenotype. Patients with MCTD have been previously reported to have an increased frequency of these HLA phenotypes when compared to healthy controls and to patients with other collagen vascular diseases.[11,52] Using these data, we suggest that our patients are clinically, serologically, histopathologically, and genetically similar to patients with MCTD and, therefore, that these cases may actually represent a forme fruste of MCTD. Although it is possible that these patients will develop other clinical manifestations of MCTD in future years, we believe that this outcome is unlikely, given the lack of development of additional symptoms over the past several years that they have been followed.

Conclusions

The importance of further analysis of the antinuclear antibody in patients with idiopathic pulmonary fibrosis without evidence of an associated collagen vascular disease is illustrated by the 3 patients in this report. It is likely that other patients with IPF who have antibodies directed against other specific nuclear antigens can be identified. Whether these cases represent a distinct subset of IPF, an incomplete form of collagen vascular disease, or a patient who is likely to develop other clinical manifestations of a more defined collagen vascular disease in future years remains to be determined. Certainly, a large prospective study to answer these questions is indicated.

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or Department of Defense.

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Accepted for publication in February, 1986. Updating, as necessary, has been done by the authors.

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