The Role of Microvascular Injury in the Evolution of Idiopathic Pulmonary Fibrosis

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Abstract

Interstitial lung disease compatible with idiopathic pulmonary fibrosis (IPF) developed in 19 previously healthy patients. Although interstitial and/or honeycomb parenchymal fibrosis was present in all, there were patchy areas of paucicellular septal capillary injury along with corroborative direct immunofluorescent evidence of a humorally mediated microvascular injury syndrome. Significantly elevated factor VIII levels were seen in 17 of 18 patients tested. Antiphospholipids were present in all 18 patients tested, comprising antibodies of phosphatidylethanolamine, beta-2 glycoprotein, phosphatidylcholine, and/or phosphatidylserine. Anti-Ro and/or anti-ribonucleoprotein (RNP) antibodies were seen in 4 patients. Serologic evidence of infection with cytomegalovirus (CMV) was found in 9 patients and parvovirus B19 (B19) in 9 patients; 1 patient was not tested. Molecular studies revealed B19 DNA in 6 of 6 B19-seropositive patients. In situ hybridization studies revealed CMV RNA in pulmonary cells in patients with serologic evidence of active CMV infection despite the absence of cytopathic changes typical of CMV infection.

Antiphospholipid antibodies, antiendothelial cell antibodies, and/or endotheliotropic viral infections related to B19 and CMV may be of pathogenetic importance to the evolution of IPF. This report underscores the potential importance of microvascular injury in the evolution of IPF.

Idiopathic pulmonary fibrosis (IPF) encompasses a distinctive subgroup of interstitial pneumonias associated with substantial pulmonary fibrosis for which the cause is obscure.1,2 The current classification scheme recognizes 4 distinct subtypes of IPF: usual interstitial pneumonitis (UIP), desquamative interstitial pneumonitis, acute interstitial pneumonitis, and nonspecific interstitial pneumonitis (NSIP). Most cases fall into the UIP and NSIP categories.1,2 The morphologic criteria for each of these entities are well defined.1 Nevertheless, the pathogenesis leading to the parenchymal changes remains elusive, and the mechanisms evoking a state of parenchymal change categorized as UIP, desquamative interstitial pneumonitis, acute interstitial pneumonitis, or NSIP are claimed to be largely unidentifiable.1

We describe the clinical, laboratory, and lung biopsy findings of 19 patients in whom pulmonary fibrosis developed in the absence of significant extrapulmonary disease; they were all categorized as having IPF. Open lung biopsy material available for all patients revealed morphologic changes compatible with UIP or NSIP according to the currently accepted criteria; however, in each case, there was septal capillary injury along with direct immunofluorescent (DIF) findings compatible with an immune-based microvascular injury syndrome. We explored the pathogenetic basis for the microvascular changes and herein describe the potential role of microvascular injury in the evolution of IPF.

Materials and Methods

Clinical Evaluation

The patients were referred to the Interstitial Lung Disease Clinic, Ohio State University Medical Center, Columbus; all
patients were assessed carefully by 2 pulmonologists (J.A. and A.P.-H.). In every case, the patient had undergone an open lung biopsy using a thorascopic video-assisted procedure by one surgeon (P.R.). In light of the pathologic findings, which in addition to manifesting classic changes of NSIP and UIP also showed septal capillary endothelial cell (EC) injury, detailed serologic testing to explore potential mechanisms of microvascular injury was conducted. Antibodies known to be associated with EC injury were evaluated and included anti-Ro(SSA), anti-ribonucleoprotein (RNP), and certain myositis-associated antibodies such as anti-Jo-1, anti-PL7, anti-PL12, anti-EJ, anti-OJ, anti-Mi-2, anti-SRP, anti-polymyositis-scleroderma (PM-Scl), anti-Ku, anti-U1RNP, anti-U2RNP, and anti-Ro. In addition, a full antiphospholipid antibody (APAB) evaluation was conducted (Table 1) in light of the proffered role of these antibodies in the induction of EC injury. The same comprehensive APAB profile was conducted in a group of 15 patients, similar in sex and age distribution to the study patients, with other forms of pulmonary disease, ie, emphysema, lymphocytic interstitial pneumonitis, pneumonia, and bronchiolitis obliterans with organizing pneumonia. Serologic evidence of parvovirus B19 (B19) and cytomegalovirus (CMV) infection were explored because both viruses have been linked pathogenetically to autoimmune microvascular disease. A factor VIII assay was performed as a quantitative index of EC injury; enhanced expression of this antigen is associated with injury of the microvasculature, especially the septal capillary bed. Factor VIII levels also were available for 7 patients with miscellaneous lung diseases in which factor VIII levels are not known to be elevated; the patient population included those with lymphocytic interstitial pneumonitis (1 patient), bacterial pneumonia (2 patients), and emphysema (4 patients).

**Indirect Immunofluorescent Rodent Lung Assay**

Serum samples also were assessed for evidence of circulating antibodies to endogenous lung components via 2 indirect assays. The first used neonatal rat lung as a substrate. After freezing the lung tissue at –60°C, it was cut at a thickness of 6 µm and placed on a slide that then was washed in phosphate-buffered saline (PBS) twice for 5 minutes. A fluorescein-conjugated goat antihuman IgG diluted at 1:20 in PBS was applied to the slide, after which it was incubated for 45 minutes and then washed in PBS twice for 5 minutes. Samples from 3 patients with other forms of lung disease (emphysema, pneumonia, adult respiratory distress syndrome) and 12 patients without known lung disease served as controls for the assay.

The second method involved the use of acetone-fixed cytocentrifuged preparations made from human pulmonary microvascular EC cultures that were incubated with a 1:100 dilution of each patient’s serum and then stained with fluorescein-conjugated goat antihuman IgG (Caltag, Burlingame, CA). Samples from 6 healthy patients served as the control.

**EC Toxicity Assay**

A direct serum EC toxicity assay using human ECs derived from umbilical vein and human pulmonary artery was conducted to determine the presence of circulating factors directly toxic to endothelium. Cells were isolated and propagated. The method has been described previously. Briefly, low-passage ECs were grown to confluence in 24-well plastic tissue culture plates (Corning Glass Works, Corning, NY), rinsed with PBS, and incubated for up to 5 days in EC growth medium in which individual patient serum samples were substituted for fetal bovine serum at a concentration of 20%. For the negative control samples, ECs were incubated in EC growth medium supplemented with normal pooled human serum. Cultures were examined microscopically for signs of stress or toxicity. All patient serum samples were tested in human umbilical vein and human pulmonary artery ECs in 2 to 4 replicate experiments.

**Pathologic Examination**

The lung tissue examined pathologically included paraffin-embedded, formalin-fixed tissue samples stained with H&E, periodic acid–Schiff, and Masson trichrome preparations. In most cases, fresh or frozen tissue for DIF and indirect immunofluorescent (IIF) testing was available: fluorescein-conjugated antibodies monospecific for IgG, IgM, IgA, C3, fibrin, and C5b-9 were applied to cryostat sections. DIF testing also was conducted on samples from 11 patients with nonimmunologic lung disorders represented by carcinoma (1 patient), an endobronchial lipoma (1 patient), emphysema (5 patients), marginal zone lymphoma (1 patient), diffuse alveolar injury (1 patient), bronchiolitis obliterans with organizing pneumonitis (1 patient), and infection (1 patient).
### Table 2
Clinical and Laboratory Features for 19 Patients With Idiopathic Pulmonary Fibrosis

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Time Course</th>
<th>Pathologic Diagnosis</th>
<th>ANA</th>
<th>Anti-ENA</th>
<th>APAB*</th>
<th>RLA</th>
<th>Viral Studies*</th>
<th>Factor VIII (%)</th>
<th>ESR (mm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/46</td>
<td>Months</td>
<td>NSIP</td>
<td>+</td>
<td>Anti-Ro</td>
<td>–</td>
<td>1</td>
<td>No result with parvovirus B19 DNA</td>
<td>ND</td>
<td>33</td>
</tr>
<tr>
<td>2/M/29</td>
<td>Months</td>
<td>UIP</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>CMV IgG and IgM, +</td>
<td>287</td>
<td>6.43</td>
</tr>
<tr>
<td>3/M/52</td>
<td>Months</td>
<td>UIP</td>
<td>–</td>
<td>–</td>
<td>Anti-beta-2 GP IgM, 24; anti-PS IgM, 24/anti-PE IgM, 2.04</td>
<td>3M</td>
<td>318</td>
<td>23.3</td>
<td>58</td>
</tr>
<tr>
<td>4/F/54</td>
<td>Months</td>
<td>NSIP</td>
<td>–</td>
<td>–</td>
<td>Anti-PC IgA, 2.50</td>
<td>Pos</td>
<td>Parvovirus B19 IgG, 5.68; parvovirus B19 DNA</td>
<td>365</td>
<td>ND</td>
</tr>
<tr>
<td>5/M/52</td>
<td>Months</td>
<td>NSIP</td>
<td>+</td>
<td>Anti-Ro</td>
<td>Anti-PE IgM, 2.46; Anti-PS IgG, 18</td>
<td>ND</td>
<td>CMV IgG; parvovirus B19 IgG, 9.89; parvovirus B19 DNA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6/M/50</td>
<td>Months</td>
<td>UIP</td>
<td>1-640</td>
<td>Anti-RNP</td>
<td>Anti-beta-2 GP IgM, 52; anti-PE IgM, 2.93; anti-PS IgM, 12</td>
<td>ND</td>
<td>Parvovirus B19 IgG, 11.5; parvovirus B19 DNA</td>
<td>ND</td>
<td>16</td>
</tr>
<tr>
<td>7/F/37 8/F/63 (died)</td>
<td>Months</td>
<td>NSIP</td>
<td>1-160</td>
<td>–</td>
<td>Anti-PE IgG, 2.74; anti-PE IgM, 2.34</td>
<td>Pos</td>
<td>CMV and parvovirus B19, –</td>
<td>255</td>
<td>19.3</td>
</tr>
<tr>
<td>9/M/62 (died)</td>
<td>2 y</td>
<td>UIP</td>
<td>–</td>
<td>–</td>
<td>Anti-beta-2 GP IgA, 70; anti-PE IgG, 1.22</td>
<td>ND</td>
<td>CMV IgG</td>
<td>367</td>
<td>98</td>
</tr>
<tr>
<td>10/F/39</td>
<td>Months</td>
<td>NSIP</td>
<td>–</td>
<td>–</td>
<td>Anti-PE IgA, 2.41; anti-PC IgA, 2.180</td>
<td>Pos</td>
<td>CMV IgG, 40.7; CMV IgM, 3.26; parvovirus B19 IgG, 8.4</td>
<td>152</td>
<td>59</td>
</tr>
<tr>
<td>11/M/61 (died)† 1 y</td>
<td>UIP</td>
<td>–</td>
<td>–</td>
<td>LA, +; anti-PE IgG, 1.60</td>
<td>Pos</td>
<td>CMV IgG, 40.7; CMV IgM, 3.26</td>
<td>157</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>12/M/57 (died)</td>
<td>1 y</td>
<td>UIP</td>
<td>–</td>
<td>–</td>
<td>Anti-beta-2 GP IgA, &gt;100; anti-PE IgA, 1.32; anti-CLP IgA, 48.10</td>
<td>Pos</td>
<td>Parvovirus B19 IgG, 1.7; parvovirus B19 DNA</td>
<td>170</td>
<td>48</td>
</tr>
<tr>
<td>13/F/33</td>
<td>Months</td>
<td>NSIP</td>
<td>–</td>
<td>–</td>
<td>Anti-CLP IgA, +</td>
<td>ND</td>
<td>Parvovirus B19, 9.2</td>
<td>High</td>
<td>ND</td>
</tr>
<tr>
<td>14/M/51 15/F/53</td>
<td>1 y</td>
<td>NSIP</td>
<td>Antinuclear/antiribonucleolar</td>
<td>–</td>
<td>Anti-Ro</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>16/M/52</td>
<td>Months</td>
<td>UIP</td>
<td>–</td>
<td>–</td>
<td>Anti-PC IgA, 1.90</td>
<td>ND</td>
<td>CMV IgG, 16.9; CMV IgM, 1.49</td>
<td>458</td>
<td>ND</td>
</tr>
<tr>
<td>17/M/64 (died)† 1 y</td>
<td>UIP</td>
<td>–</td>
<td>–</td>
<td>Anti-PE IgG, 2.36; anti-PE IgM, 2.02; anti-PE IgA, 3.84; indeterminate LA</td>
<td>ND</td>
<td>CMV IgG, +</td>
<td>287</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td>18/M/57 19/F/46</td>
<td>Months</td>
<td>UIP</td>
<td>–</td>
<td>–</td>
<td>Anti-beta-2 GP IgM, 42</td>
<td>Pos</td>
<td>CMV IgG, &gt;500; parvovirus B19 DNA</td>
<td>458</td>
<td>ND</td>
</tr>
</tbody>
</table>

ANA, antinuclear antibodies; anti-CLP, anticardiolipin; anti-ENA, anti-extractable nuclear antigen antibodies; anti-PC, antiphosphatidylcholine; anti-PE, antiphosphatidylethanolamine; anti-PS, antiphosphatidylserine; APAB, antiprophospholipid antibody; CMV, cytomegalovirus; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GP, glycoprotein; GrNuc, granular nuclear; IPCA, indirect pulmonary cytocentrifuged assay; LA, lupus anticoagulant; ND, not done; NSIP, nonspecific interstitial pneumonitis; Pos, morphologic evidence of apoptosis; RLA, rodent lung assay; RNP, ribonucleoprotein; UIP, usual interstitial pneumonitis; +, positive; −, negative.

* V alues given are conventional units. To convert the conventional values for IgA, IgG, and IgM (mg/dL) to Système International (SI) units (g/L), multiply by 0.01; the SI values for factor VIII (proportion of 1.0) also are obtained by multiplying by 0.01. See Table 3 for the reference ranges for selected variables.

† Underwent unilateral transplantation.

### Table 3
Reference Ranges*

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Equivocal</th>
<th>Weak Positive</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>&lt;1.07</td>
<td>1.08-1.62</td>
<td>1.63-2.17</td>
<td>&gt;2.17</td>
</tr>
<tr>
<td>IgM</td>
<td>&lt;1.77</td>
<td>1.78-2.49</td>
<td>2.50-3.21</td>
<td>&gt;3.22</td>
</tr>
<tr>
<td>IgA</td>
<td>&lt;2.67</td>
<td>2.68-3.66</td>
<td>3.67-4.66</td>
<td>&gt;4.67</td>
</tr>
<tr>
<td>IgG</td>
<td>&lt;12.22</td>
<td>12.23-17.06</td>
<td>17.07-21.89</td>
<td>&gt;21.90</td>
</tr>
<tr>
<td>IgM</td>
<td>&lt;6.72</td>
<td>6.73-10.10</td>
<td>10.11-13.49</td>
<td>&gt;13.50</td>
</tr>
<tr>
<td>IgG</td>
<td>&lt;12.22</td>
<td>12.23-17.06</td>
<td>17.07-21.89</td>
<td>&gt;21.90</td>
</tr>
<tr>
<td>IgM</td>
<td>&lt;6.72</td>
<td>6.73-10.10</td>
<td>10.11-13.49</td>
<td>&gt;13.50</td>
</tr>
<tr>
<td>IgA</td>
<td>&lt;1.63</td>
<td>1.64-2.44</td>
<td>2.45-3.25</td>
<td>&gt;3.26</td>
</tr>
<tr>
<td>IgG</td>
<td>&lt;20</td>
<td>—</td>
<td>—</td>
<td>≥20</td>
</tr>
<tr>
<td>IgM</td>
<td>&lt;15</td>
<td>—</td>
<td>—</td>
<td>≥15</td>
</tr>
<tr>
<td>IgA</td>
<td>&lt;20</td>
<td>—</td>
<td>—</td>
<td>≥20</td>
</tr>
</tbody>
</table>

* Units are arbitrary units from an international standard where 1 GPL unit = the binding activity of purified IgG at 1 µg/mL, 1 MPL unit = the binding activity of purified IgM at 1 µg/mL, and 1 APL unit = the binding activity of purified IgA at 1 µg/mL.
Molecular Studies

Tissues were examined for evidence of CMV protein expression via in situ reverse transcriptase polymerase chain reaction (PCR) method and B19 DNA via solution phase PCR; the nature of the test run (ie, CMV vs B19) was based on the serologic data. The techniques used for the determination of CMV DNA and B19 DNA have been described previously. As a control comparison, CMV in situ studies were conducted on normal lung tissue removed as a part of a lobectomy procedure for underlying carcinoma, while B19 DNA analysis was conducted on normal lung tissue samples from 9 patients, amniotic fluid (2 samples), liver (2 samples), and brain (1 sample). The samples were obtained from patients without serologic confirmation of B19 infection.

Results

Clinical and Selected Laboratory Test Findings

The clinical findings and selected laboratory test results are summarized in Table 2. The patient population comprised 8 women and 11 men (mean age, 50.9 years). In each case, the patients sought care because of a relatively rapid or more insidious onset of shortness of breath; the clinical course ranged from 2 months to 1 year before undergoing open lung biopsy. In all cases, the clinical diagnosis was IPF based on a careful integration of clinical and radiographic features. Serologic testing revealed antinuclear antibodies in 5 of 18 patients, with antibodies to Ro in 3 and to RNP in another, despite the absence of any features indicative of a connective tissue disease (CTD) diathesis. The APAB profile was positive in all patients tested. In 4 patients there was a positive lupus anticoagulant. In addition, the C-reactive protein concentration was elevated in 9 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested. The erythrocyte sedimentation rate was elevated in 9 of 10 patients tested.

Viral testing was conducted in all patients except 1. There was evidence of active CMV infection by virtue of CMV IgM antibodies in 6 patients (cases 2, 3, 8, 10, 11, and 16). In addition, cases 5, 17, and 18 had high CMV-specific IgG antibody levels, but in the absence of CMV-associated IgM. High levels of IgG B19 antibodies (>5 mg/dL), held to be the defining profile indicating chronic persistent infection, were detected in 9 patients tested, with IgG titers ranging from 5.68 to 11.5, excluding cases 12 and 19 in whom the IgG titers were 1.70 and 2.20, respectively. One patient was not tested.

Seven patients (cases 2, 3, 10-13, and 17) had a rapidly progressive clinical course that was refractory to cyclophosphamide therapy, resulting in unilateral lung transplantation. Five patients (cases 8, 9, 11, 12, and 17) died despite aggressive immunosuppressive therapy. The remaining patients remain symptomatic, although their conditions seem stable.

The assessment of anti–beta-2 glycoprotein, antiphosphatidylserine, antiphosphatidylethanolamine, and antiphosphatidylcholine was conducted in the control group; only 3 of 15 patients had antibody levels at significantly detectable levels. These antibodies were detected at a significantly higher rate in the study group than in the control group ($P < .002$). The factor VIII levels in the control group ranged from 1 to 136 (mean value, 96).

Pathologic Findings

All biopsy specimens showed varying degrees of inflammation and fibrosis of the alveolar walls. In 9 cases there was uniform expansion of the alveolar walls by eosinophilic collagen with minimal accompanying lymphocytic and plasmacellular inflammation. Although there were zones of uninvolved lung parenchyma, the changes were temporally uniform. The findings fulfilled morphologic criteria to warrant the designation of NSIP (cases 1, 4, 5, 7, 8, 10, and 13-15) Image 1. In the other 9 cases, there was considerable heterogeneity in the parenchymal changes with alternating zones of honeycomb parenchymal fibrosis, septal fibroplasia, focal inflammation, and uninvolved lung parenchyma. Furthermore, the septal fibrosis demonstrated histologic variability with some foci demonstrating paucicellular, fibroblast-poor eosinophilic fibrosis, while other areas showed prominent myofibroblastic proliferation, mucin deposition, and delicate collagenosis Image 2. These remaining cases (cases 2, 3, 6, 9, 11, 12, and 16-19), therefore, fulfilled the accepted morphologic criteria for UIP.

Although all cases could be categorized as representing NSIP or UIP, there was morphologic evidence of septal capillary injury characterized by RBC extravasation and hemosiderin deposition as confirmed on an iron stain Image 3 and Image 4. Higher power examination revealed one or more of the following specific capillary alterations: (1) fibrin...
deposition in one or more of the intra-alveolar spaces, septal capillary walls, and/or lumens; (2) endothelial denudement, with some vessels manifesting complete EC denudement [Image 5]; and (3) basement membrane zone reduplication and corrugation (Image 5), which imparted a double contour to the basement membrane zone, best visualized with periodic acid–Schiff stain.

The DIF and IIF studies revealed granular deposition of IgM, IgG, and IgA within the septal capillaries, in concert with septal capillary C3 and C5b-9 deposition in all cases. In some cases there was nuclear EC staining corresponding to antibodies to any one of the extractable nuclear antigens (eg, anti-Ro, anti-RNP) [Image 6]. Prominent vascular IgA deposition was observed in the cases in which an antiphospholipid antibody of the IgA isotype was found. The IIF showed weak to moderate EC staining of septal capillaries in 8 cases that included all 3 cases in which there was a positive antinuclear antibody; in 6 cases there was no staining. The indirect cytocentrifuged assay using pulmonary microvascular endothelium as a substrate revealed prominent granular nuclear staining of many of the cells [Image 7] in cases 2, 3, 4, 7, 10, 11, and 12 and was negative in case 9. There was no significant difference in the immunofluorescent profile between patients with an NSIP pattern and those with UIP morphologic features. Results of the DIF and IIF studies and those for the indirect cytocentrifuged assay in the control patients were negative.

In patients in whom CMV serologic results were positive, in situ hybridization studies of lung biopsy specimens for CMV RNA revealed focal positivity in endothelia, pneumocytes, and alveolar macrophages. Expression was nuclear and/or cytoplasmic and weak in intensity; concomitant viral cytopathic changes were not observed. Solution phase PCR revealed B19 DNA in the lung tissue of all 6 patients tested [Image 8], in whom serologic results corroborated recent infection based on markedly elevated B19 IgG-specific antibodies.

The CMV in situ assay conducted on normal lung tissue fragments from patients without known positive CMV serologic
test results was negative. The solution phase B19 assay conducted on various extrapulmonary specimens and on 2 normal lung tissue samples was negative. Seven additional normal lung tissue samples had no amplifiable DNA, and, therefore, the assay was deemed unsuccessful.

In cases 2, 3, 8, and 11, the pulmonary and umbilical vein and fibroblast toxicity assays showed progressive loss of endothelia. In cases 8 and 11, there was complete destruction of monolayers within 20 hours. In case 2, there was progressive loss of endothelia over 48 hours. In case 3, the ECs exhibited features suggestive of a stressed state, ie, progressive spindled morphologic features with enhanced cytoplasmic granularity over a period of 48 to 72 hours. In the other cases there were no significant changes of endothelia after 5 days of incubation with the culture substrate.
The pooled human serum samples demonstrated no evidence of toxicity or morphologic evidence of apoptosis.

Discussion

We describe 19 cases in which the patients sought care because of signs and symptoms of interstitial lung disease that were clinically and morphologically compatible with IPF and confirmed by lung biopsy. In almost every case, the clinical and pathologic impression was consistent with UIP or NSIP.

Pulmonary fibrosis is a process characterized by the deposition of excessive collagen within the terminal lung parenchyma, often preceded by an inflammatory phase. In 15% of cases, the process may be linked directly with a known CTD. In other cases, there is an association between an inhaled fibrogenic agent such as silica, asbestos, or an organic allergen, the latter producing a syndrome of hypersensitivity pneumonitis. However, there is a subgroup of patients with pulmonary fibrosis in whom a specific cause has not been established. These patients fall under the general rubric of IPF. Alternative appellations include cryptogenic fibrosing alveolitis and idiopathic interstitial pneumonia. The entity of IPF was first recognized in 1944 by Hamman and Rich, who described a series of 4 patients manifesting a rapidly progressive respiratory illness that terminated in death. They suggested that a single cause of this syndrome might be implicated, although one could not be clearly identified. The 2 most common subtypes of
idiopathic interstitial pneumonia are UIP and NSIP. UIP portends the poorest prognosis, with a median survival of only 2.5 to 3.5 years, and generally has a poor response to treatment. NSIP has more variable manifestations, course, and response to treatment, but the prognosis generally is much better than that of IPF, with some patients experiencing full recovery. Estimates of prognosis with NSIP may be more accurate when radiographic and pathologic features are considered. More important, the pathologic finding of NSIP should prompt a careful search for the underlying cause, specifically in the context of collagen vascular disease. In our study, patients with a UIP picture had a more accelerated clinical course than those with NSIP, leading to lung transplantation within less than a year from diagnosis and associated with death in 5 patients within less than 2 years from diagnosis.

Much of our current understanding of the pathogenesis of IPF is derived from animal models. Regardless of the inciting injurious trigger, animals manifest reproducible stereotypic morphologic features characterized by hemorrhage with evidence of capillary EC injury and concomitant type I alveolar cell injury followed by proliferation of type II alveolar cells and ensuing fibroplasia. While animal models have focused on EC injury, and EC injury is described in cases of fibrosing alveolitis associated with CTD and viral pneumonia, clinical studies of IPF rarely allude directly to a potential role for microvascular injury in the induction of fibrosis. Of interest, however, are 3 articles that describe antibodies to cytokeratins 8, 18, and 19 in patients with IPF. These particular cytokeratins also are expressed uniquely by pulmonary microvascular septal capillary endothelium. While the published studies allude to the importance of these antibodies in the context of humoral immunity directed against pulmonary epithelial cell constituents, it is in the realm of possibility that the microvascular EC bed constitutes the true antigenic target.
The nature of the microvascular changes, ie, fibrin, minimal inflammation, and EC denudement, were typical for toxic and/or immune-based EC injury syndromes.\(^4,34\) Serologic evidence supportive of substantial microvascular injury in these patients was the markedly elevated factor VIII levels. Serologic factor VIII levels are widely accepted in the rheumatology literature as an index of EC injury.\(^35\)

An immune-based cause for the microvascular changes was suggested by the DIF and IIF studies that revealed a pattern characteristic of humorally mediated microvascular injury. With respect to the DIF findings, there was deposition of C3, C5b-9, IgG, IgA, and IgM within the pulmonary microvasculature, consistent with in vivo activation of the classic complement cascade sequence.\(^3,4,34\) Furthermore, both IIF assays using neonatal rat lung and pulmonary microvascular EC cultures as substrates showed fine granular staining of endothelium, corroborative of the presence of an anti-EC antibody.

The question arises regarding the basis of the humorally mediated microvascular injury. While none of our patients fulfilled the criteria for CTD, 4 had antibodies to Ro, and 1 patient had antibodies to RNP. Furthermore, in these patients, DIF studies of the lung showed nuclear staining of the septal capillary endothelium for IgG and C5b-9, with a similar staining pattern seen via the IIF assay, consistent with a Gell and Coombs type II immune reaction directed at endothelium.

EC injury as a mechanism of inducing pulmonary fibrosis has been demonstrated in the setting of select autoimmune CTD syndromes, viral pneumonia, and idiopathic pulmonary hemosiderosis.\(^36-40\) Patients with CTD in whom fibrosing pneumonitis develops characteristically have anti-ENA antibodies, especially anti-Ro/SSA or anti-RNP and/or one of the myositis-associated antibodies such as anti-Jo-1.\(^41-45\) The basis of the microangiopathy has been postulated to reflect an anti-EC antibody with C5b-9 as the effector mechanism. It is suggested that endothelia demonstrate up-regulated ENA expression owing to the displacement of nuclear or cytoplasmic ENA to the cell surface,\(^30\) a classic trigger being accelerated apoptosis. In vitro models have been established that demonstrate cytotoxic effects of anti-Ro/SSA AB on endothelium.\(^46\) While the association of anti-Ro antibodies, anti-RNP antibodies, or both with the development of pulmonary fibrosis is well established, the mechanisms by which these antibodies induce fibroplasias has not been explored. There are other reports in the literature linking myositis-associated antibodies and anti-Ro/SSA antibodies to idiopathic interstitial fibrosis. One study describes 5 patients with interstitial lung disease in the setting of antibodies to the aminocyl-tRNA synthetases\(^47\) in the absence of clinical stigmata of collagen vascular disease. A second describes anti-Ro/SSA–associated pneumonitis without clinical evidence of lupus erythematosus.\(^48\)

Another factor possibly contributing to the microvascular injury was the presence of 1 or more antiphospholipid antibodies. Although antiphospholipid antibodies typically are held to evoke microvascular changes primarily in the context of vascular thrombosis, it is becoming increasingly apparent that antiphospholipids may be associated with direct EC injury via acceleration of EC apoptosis and/or in the context of the endothelium as the immunogen.\(^5-7\) The main implicated antibodies in our series, ie, the more unusual antibody subtypes directed against surface cytoplasmic-based antigens (ie, antiphosphatidylserine, antiphosphatidylcholine, antiphosphatidylethanolamine, and anti–beta-2 glycoprotein), have been correlated strongly with EC injury mediated through accelerated apoptosis.\(^5-7,49-59\)

The EC toxicity assays in 4 of our patients showed morphologic evidence of apoptosis. Further corroboration of a direct role for the antibodies in the induction of microvascular injury is the finding by DIF of immunoglobulin deposition mirroring the specific APAB isotype. For example, all cases in which IgA-associated APABs were found had prominent IgA deposition in the septal vasculature. Second, both the IIF and DIF studies in a few patients showed nuclear and/or cytoplasmic staining of the endothelium for IgG. One study demonstrated anti-EC binding activity to a range of cell membranes in patients with primary APAB syndrome.\(^59\) In our study, IIF studies showed anti-EC antibody activity.
whereby the only known antibody was one directed to a phospholipid, beta-2 glycoprotein, or both, which is supportive of this concept. Nevertheless, it has not been concluded with any certainty that the true effector mechanism of EC injury is the antiphospholipid per se. Specifically, other anti-EC antibodies or other mechanisms such as an endotheliotropic viral infection may lead to accelerated apoptosis with resultant displacement of select surface phospholipids evoking a state of neoantigenicity to which antibodies are formed as a secondary epiphenomenon (discussed below). Such antibodies are really a marker of the disease as opposed to being the primary mechanism evoking the state of tissue injury.

Regarding a potential role for a viral trigger, B19 DNA was recovered from the lung biopsy specimens of patients with elevated anti-B19 antibodies, while CMV RNA was detected in the lung biopsy specimens from patients with serologic evidence of active or recent CMV infection. Both CMV and B19 are endotheliotropic and are implicated etiologically in CTDs in which microvascular injury is held to be important to lesional pathogenesis, ie, scleroderma, mixed CTD, and dermatomyositis, as well as in multiorgan vasculitic syndromes including microscopic polyarteritis nodosa and Wegener granulomatosis. The mechanisms by which B19 induces autoimmune vascular disease remain speculative; up-regulation of the tumor necrosis factor alpha receptor pathway leading to accelerated apoptosis is thought to be critical. With respect to CMV, the viral infection in our patients was in the context of low-level reactivation or latent infection occurring in an immunocompetent host unassociated with the classic cytopathic changes of CMV infection. While fully expressed CMV infection is antia apoptotic for endothelia via the mechanism of cytoplasmic sequestration of p53 accelerated apoptosis of endothelium can occur in the setting of latent infection or low-level infectivity unassociated with cytopathic change. It also is interesting to note that the antibodies formed in our patients, ie, select uncommon antiphospholipids, and anti-Ro and anti-RNP antibodies are all formed in the setting of accelerated apoptosis, suggesting a potential link between the viral infection and the unique autoantibody profile characteristic of patients with IPF.

We have described a distinctive group of patients with IPF that could be categorized as representing NSIP or UIP; an immune-based septal capillary EC injury syndrome in concert with a highly characteristic and reproducible serologic profile including the presence of specific antiphospholipid antibodies and antibody serologic results indicative of chronic persistent viral infection emerged. A summary of the standard panel to study patients with pulmonary fibrosis is given in Table 1. The presence of a profile comparable to the one encountered in our study group patients would suggest a diagnosis of IPF even in the absence of a confirmatory open lung biopsy. We believe that microvascular injury may be important in the evolution of IPF through the aforementioned mechanism.

References


