Acute Pulmonary Complications in Systemic Lupus Erythematosus

Immunofluorescence and Light Microscopic Study

LOUIS P. PERTSCHUK, D. O., LOUIS F. MOCCIA, M.D., YALE ROSEN, M.D., HAROLD LYONS, M.D., CATHERINE M. MARINO, M.D., ALLEN A. RASHFORD, M.D., AND CHRISTINE M. WOLLSCHLAGER, M.D.

Pertschuk, Louis P., Moccia, Louis F., Rosen, Yale, Lyons, Harold, Marino, Catherine M., Rashford, Allen A., and Wollschlager, Christine: Acute pulmonary complications in systemic lupus erythematosus. Immunofluorescence and light microscopic study. Am J Clin Pathol 68: 553-557, 1977. Lung tissue obtained from eight consecutive patients with systemic lupus erythematosus complicated by severe, acute pulmonary disease was studied by both light and immunofluorescence microscopy. Light microscopic examination disclosed interstitial pneumonia in four cases, cytomegalovirus pneumonitis in one case, bronchiolitis and peribronchiolitis in one case, pulmonary infarction in one case and focal atelectasis in the remaining case. Direct immunofluorescence examination revealed focally bound immunoglobulins or complement (C3) within pleural and/or pneumocyte nuclei in each specimen. Immunohistologic studies in these cases may thus suggest a diagnosis of systemic lupus erythematosus with acute pulmonary complications, despite the lack of specificity of the pathologic changes seen by light microscopy. (Key words: Systemic lupus erythematosus; Pulmonary complications; Immunofluorescence; Immunopathology.)

DURING THE COURSE of systemic lupus erythematosus (SLE), patients are more apt to manifest evidence of pulmonary or pleural disease than are those afflicted with other connective tissue diseases.1,2 Reported incidences range from 50 to 70%.3,4 The usual cause of pulmonary disease in SLE is bacterial, tuberculous or mycotic infection.2,7 In a small number of patients, where the etiology of the pulmonary disease is unclear, there are problems in both diagnosis and management, and such patients often develop symptoms with alarming rapidity. Ventilatory insufficiency with respiratory failure may quickly supervene, and a tissue diagnosis is then of utmost value.

We report the pathologic and immunopathologic findings in eight cases of SLE with severe pulmonary complications, clinically known as "acute lupus pneumonitis,"8 seen at this center during the past 12 months.

Materials and Methods

Patients

The group studied was composed of seven women and one man. Presenting complaints were dyspnea, cough and chest pain of 1-2 days' duration. All had the diagnosis of SLE made according to criteria adopted by The American Rheumatism Association.4

Two patients were not known to have SLE prior to admission to the hospital. The diagnosis of SLE had been established in the other cases for periods ranging from 4 months to 7 years. In all cases there were clinical evidences of multisystem involvement, elevated antinuclear antibody (ANA) titers, and positive LE cell preparations.
In seven cases, pleural or open lung biopsy was performed. This was necessitated by deterioration in the patient’s clinical condition, and an inability to diagnose the nature of the pulmonary disease specifically despite extensive studies. The eighth patient died shortly after admission to the hospital. Postmortem material was available from the latter case, as well as from two of the patients previously biopsied.

Pathologic Studies

Tissues were obtained at thoracotomy, or at autopsy as soon as possible after death (8–24 hours). Material was submitted for bacterial, fungal and Mycobacterium tuberculosis cultures. A portion of each specimen was rapidly immersed in liquid nitrogen for immunofluorescence examination. Tissue for light microscopy was fixed in 10% buffered formalin and routinely embedded in paraffin. The following staining methods were employed: hematoxylin and eosin, periodic acid–Schiff, Gomori methenamine silver, Ziehl-Neelsen, and Gram.

Immunofluorescence Studies

Frozen sections 4 µm thick were processed for direct immunofluorescence examination using standard

<table>
<thead>
<tr>
<th>Case</th>
<th>Tissue Source</th>
<th>Histology</th>
<th>Pleura</th>
<th>Parenchyma</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>IgE</th>
<th>C3</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Surgical</td>
<td>Infarct, organizing pneumonia</td>
<td>Pos.</td>
<td>Neg.</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Surgical</td>
<td>Desquamative interstitial pneumonia</td>
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<td>Neg.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Autopsy</td>
<td>Interstitial pneumonia</td>
<td>Neg.</td>
<td>Pos.</td>
<td>0</td>
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<td>+</td>
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<tr>
<td>4</td>
<td>Surgical</td>
<td>Interstitial pneumonia</td>
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<td>Pos.</td>
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<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Surgical</td>
<td>Bronchiolitis, peribronchiolitis</td>
<td>Pos.</td>
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<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Pleural biopsy</td>
<td>No significant findings</td>
<td>Pos.</td>
<td>Neg.</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Autopsy</td>
<td>Focal atelectasis, hemorrhage</td>
<td>Pos.</td>
<td>Pos.</td>
<td>0</td>
<td>+</td>
<td>0</td>
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<tr>
<td>8</td>
<td>Surgical</td>
<td>Interstitial pneumonia, pleuritis</td>
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<td>Pos.</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>8</td>
<td>Surgical</td>
<td>Cytomegalovirus</td>
<td>Neg.</td>
<td>Neg.</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>8</td>
<td>Autopsy</td>
<td>Cytomegalovirus</td>
<td>Pos.</td>
<td>Neg.</td>
<td>+</td>
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</tbody>
</table>

* = pleural biopsy only; 0 = no fluorescent nuclei seen; + = fluorescent nuclei present.
FIG. 3. Lung, Case 7, showing prominent nuclear fluorescence of the alveolar lining cells. Antihuman complement (C3) fluorescein conjugate. ×100.

technics. Goat antihuman fluorescein conjugates specific for IgG, IgM, IgA, IgE, albumin, and C3 were employed. The molar fluorescein-to-protein ratios of these conjugates were 3.5, 3.9, 3.3, 2.4, 2.1 and 2.3, respectively. Each was diluted with phosphate-buffered saline solution containing 4% bovine serum albumin, to reach a working solution containing 50 μg/ml specific antibody, and were overlaid on tissue sections for 30 min after an initial 15-min buffer wash. After a final 60-min wash, processed slides were examined by incident light with a Zeiss Universal Research microscope equipped with an Osram HBO-200 high-pressure mercury vapor lamp, an FITC interference–excitation filter, and a K530 barrier filter.

Substrate controls consisted of lung tissue obtained at surgery or at autopsy. These specimens were from patients of similar age and sex distribution with a variety of pulmonary diseases including sarcoidosis (10 cases), pulmonary fibrosis (4 cases), bronchiolitis obliterans (12 cases) and various lung tumors (16 cases). Specimens of pulmonary tissue from five renal transplant recipients and two hepatic transplant recipients who came to autopsy were included, as well as lung tissue obtained from 15 patients who had SLE at the time of death, but did not have clinical or pathologic evidence of acute pulmonary disease.

Serum controls included unlabeled antiserum to immunoglobulins and to C3. In addition, the use of multiple conjugates provided mutual specificity controls.

Results

Pathologic Studies

Microscopic examination disclosed a variety of findings similar to those reported elsewhere (Table 1).

In Case 1, there were organizing and partially recanalized pulmonary thromboemboli with extensive infarction of pulmonary parenchyma. Fibrinous pleuritis was prominent over the infarcted area. An organizing pneumonia at the periphery was probably a secondary reaction to the infarct. Specimens from Case 2 had a morphologic appearance of a desquamative interstitial pneumonia. One small focus of nonspecific pleural inflammation was noted. Material from Case 3 was examined at autopsy only and revealed nonspecific interstitial pneumonia with intra-alveolar hemorrhage and prominent hyaline membranes. Case 4 also showed a nonspecific interstitial pneumonia with a focal desquamative pattern characterized by sheets of large mononuclear cells, probably macrophages, in the alveoli. In Case 5 there was widespread bronchiolitis with peribronchiolitis. Lymphoid follicles with germinal centers were seen in the bronchiolar walls and a purulent exudate was present within the lumens. The pleura was unremarkable.
An antemortem pleural biopsy in Case 6 was normal. At autopsy, the lungs evidenced only slight focal atelectasis and hemorrhage. Case 7 showed a non-specific, focally desquamative pneumonia and a fibrinous pleuritis. In Case 8, there was extensive cytomegalovirus pneumonitis in both biopsy and autopsy specimens. The pleura appeared unremarkable.

Microorganisms were not detected in any of the tissues examined, and no viral inclusions were identified except in Case 8. Cultures were negative in all cases.

**Immunofluorescence Studies**

Direct immunofluorescence examination revealed focal nuclear staining of pleural mesothelium and/or alveolar lining cells with a variety of conjugates in every case (Table 1, Figs. 1–4). No deposit of immunoglobulin or C3 was seen in the interstitium. IgG was deposited in arterioles in Case 8. Blocking controls were negative. Nuclear fluorescence was not seen in the control specimens. Alveolar lining cell fluorescence, where present, was thought to occur principally in type II pneumocytes.

Fluorescence was limited to the nuclei of mesothelial cells in five cases and to pneumocyte nuclei in two cases, and was detected in both locations in one case. In Case 8, fluorescent mesothelial nuclei were not detected in the biopsy specimen but were visible in the autopsy specimen. The nuclei were always diffusely stained.

**Discussion**

Patients who have SLE and acute, severe pulmonary complications have a rapidly deteriorating clinical course, and may manifest tachypnea, dyspnea, cyanosis, pleuritic chest pain, tachycardia, and often, fever. Physical findings are often minimal and may be limited to scattered rales in the lungs. Chest roentgenograms usually show basilar pulmonary infiltrates. None of these signs or symptoms are specific.

An aggressive diagnostic approach is warranted in these cases in order that specific pathologic processes can be recognized or excluded. Case 1 illustrates the benefits to be gained from open lung biopsy. In this case, pathologic examination revealed the presence of pulmonary infarction, enabling successful treatment with anticoagulants.

Previous studies of the lungs of patients with SLE by immunofluorescence have produced scant information. Alveolar deposits of gamma-globulin were detected in one patient with SLE and alveolar-wall fibrosis, and mesothelial fluorescence has been seen in pleural fluid removed from one patient who had an effusion and high antinuclear antibody titer. Gr

Granular fluorescence has been observed in a necrotic, inflammatory area in the lung in another case of SLE. Homogenates of lung reportedly were positive for antinuclear antibody in 12 of 17 cases of SLE.

To our knowledge, nuclear fluorescence of pleural or alveolar lining cells has not previously been shown to be a consistent immunopathologic finding in patients with SLE complicated by severe, acute pulmonary disease.

The presence of nuclear staining in any direct immunofluorescence preparation of tissues removed from patients with SLE (skin, kidney) is usually regarded with suspicion. Such a finding, uncommon as it may be, is usually attributed to a reaction of nuclei with antinuclear antibody, present within the specimen, occurring after its removal, or after death, i.e., an artefactual, in vitro process. If such a supposition were correct, one would anticipate that it would be a more common phenomenon, especially in autopsy material. However, we have not seen nuclear fluorescence in the lung tissue of any patient with SLE studied at autopsy, except as reported here, even when the antinuclear antibody titer was more than 1:1,000. We have not seen this phenomenon in examining other organs and tissues removed from such patients, even when necropsy was delayed more than 24 hours, except in one case where focal nuclear staining of renal tubule cells was present. Additionally, one might expect that artefactual staining would appear at the edges of a specimen, or throughout the specimen, rather than being focal.

The non-artefactual nature of nuclear staining in this study is further supported by the variety of immunoglobulin classes detected, in contrast to the usual antinuclear antibody in SLE, which is predominantly IgG. The presence of C3 in half of the specimens is especially unlikely to be an in-vitro phenomenon. We cannot, however, entirely dismiss the possibility that an in-vitro reaction between antigen and antibody occurred, although the likelihood that this should happen in eight consecutive cases seems remote.

We have attempted, without success, to reproduce nuclear staining by allowing lung, renal and cutaneous tissue removed from 20 NZB/W hybrid mice with high antinuclear antibody titers and advanced murine lupus to remain at room temperature for several hours prior to freezing. Paradoxically, pleural nuclear fluorescence was observed in two of 20 littersmates when lung tissue was frozen in liquid nitrogen within a few minutes after sacrifice. These experimental findings support the hypothesis that an in-vivo reac-
tion between nuclei and antibody may occasionally occur.

It is obvious that the clinical entity known as acute lupus pneumonitis may be produced by a variety of pathologic processes. The histologic picture in those patients with interstitial pneumonitis was nonspecific and similar to that which may be seen in viral pneumonia, uremia, or oxygen toxicity. None of our four patients who had this pathologic change was severely uremic or had received prolonged, high concentrations of oxygen. Viral studies were not performed; however, viral inclusions were not seen in these cases.

The unusual immunofluorescent findings common to all cases may have resulted from damage to cell membranes, possibly induced by an infective agent, infarction, or uremia, which allowed antibody access to antigen. Admittedly, it is difficult to conceive of such injury predominantly affecting pleural mesothelium. Of interest is the fact that the patient who had the least pulmonary disease by light microscopy (Case 6) had the highest level of blood urea nitrogen (180 mg/dl; 64.3 mmol/l) and the most strongly positive fluorescence (Fig. 2). It is possible that the findings in this case represented the residue of pre-existing pulmonary disease mostly resolved at the time of death. Antemortem cytologic studies of this patient’s pleural fluid had disclosed atypical mesothelial cells similar to those previously reported to be present in patients with SLE.11 The prominent fluorescence of mesothelial nuclei both on biopsy and at autopsy suggests that immunofluorescence studies of the pleural fluid from patients with effusion and SLE might be rewarding.

Postmortem examination of renal tissue was performed in three cases. In all specimens deposits of IgM and C3 were present in the glomerular capillary loops. One patient (Case 8) additionally had IgG deposits. Thus, there was poor correlation as to the class of immunoglobulin deposited in lung and kidney in two cases.

Although the pathogenesis underlying the phenomenon of in-vivo binding of immunoglobulins and C3 to the nuclei of mesothelial cells and pneumocytes is obscure, nonetheless the immunopathologic findings are sufficiently unusual to suggest a diagnosis of SLE with acute pulmonary complications, even though the findings on light microscopic examination are not specific.

References