Legionnaires' Disease Pneumonia: Histopathologic Features and Comparison with Microbial and Chemical Pneumonias*

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ABSTRACT

The histopathologic findings in lung tissue are reported from five cases of Philadelphia Legionnaires’ Disease and the results are compared to pneumonias caused by other microbial and chemical agents. Histopathology of lung tissue was similar in all cases, despite the fact that death occurred between the fourth and 14th day of clinical illness. The inflammatory response was almost totally limited to the lower respiratory tract and primarily involved respiratory bronchioles, alveolar ducts and alveoli. Major bronchial branches and pulmonary interstices showed little or no involvement. There was considerable variation in the extent and nature of the consolidation, but the overall reaction pattern was highly characteristic of diffuse alveolar damage. Most involved areas showed intra-alveolar, fibrinocellular mononuclear cell predominant exudates, associated with pneumocytic hyperplasia and slough. These findings plus the presence of erythroleucocytosis by macrophages and paucity of polymorphonuclear leucocytes are commonly associated with psittacine pneumonia, and much less so with classic patterns of bacterial, viral, fungal or rickettsial pneumonias. Of the toxic inhalants, nickel carbonyl, phosgene, nitrous oxide, cadmium oxide and some halogenated hydrocarbons have been associated with this tissue reaction pattern. Bacteria were notably absent in lung tissue stained by methods used to demonstrate the Legionnaires’ Disease agent.

Introduction

An unusually severe pneumonia, Legionnaires’ Disease (LD), resulting in 182 cases and 29 deaths, occurred in Philadelphia during July and August of 1976. After five months of intensive epidemiologic and laboratory investigation, the Center for Disease Control (CDC) reported the isolation of a Gram-negative bacterium from lung tissue of disease victims and identified it as the putative agent by demonstrating immunofluorescent antibodies in serum of patients surviving the illness. Findings in the lungs of 14 autopsied victims were initially reported as non-specific and probably produced by a toxic substance.
Most showed areas of extensive pneumonitis with some areas of mild interstitial reaction. Special stains revealed no fungi, bacteria or other microorganisms except in one case with disseminated candidiasis. More recently, the pulmonary tissue reaction pattern was described as "similar to those associated with a lobar pneumonia," with acute fibrinopurulent pneumonia in 21 cases and diffuse alveolar disease in 13 cases. In 19 of 26 LD victims, bacilli were readily demonstrated in lung tissue by the Dieterle silver-impregnation method and found inconsistently with conventional histopathologic stains for microorganisms.

The epidemiologic features and clinical findings of progressive atypical pneumonia associated with fever and relative bradycardia, with apparent response to tetracycline therapy, suggest the clinical diagnosis of a zoonosis, particularly psittacosis. Furthermore, our initial study of lung tissue did not reveal changes seen in acute bacterial pneumonia. Instead, the present authors found a fibrinocellular, mononuclear cell predominant, intra-aveolar exudative process more commonly described in psittacosis, but also seen in toxic, viral, fungal and mycoplasma pneumonia. To compare, in further details, the histopathologic features of these clinically similar entities, tissue sections were studied from five fatal LD cases and from two fatal cases of psittacosis and our findings were compared with those seen in other microbial or toxic pneumonias.

**Material and Methods**

In table I are given the sex, age, days of clinical illness at the time of death, underlying disease process, antibiotic therapy and summary of the gross description of the lung noted at the time of autopsy. All cases were listed as LD victims by the

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age Yr/</th>
<th>Day of Illness</th>
<th>Cause of Death</th>
<th>Underlying Disease</th>
<th>Antibiotic Therapy</th>
<th>Autopsy Findings</th>
<th>Gross Description of Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41/M</td>
<td>5</td>
<td>Acute Pneumonitis</td>
<td>Diabetes Mellitus</td>
<td>Penicillin</td>
<td>Plum colored pleural surface. Fibrinous pleuritis. Diffuse consolidated - all lobes. Appearance of red hepatization on cut surface.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>55/F</td>
<td>6</td>
<td>Bilateral Pneumonia</td>
<td>ASCVD* Angina Pectoris Old myocardial infarct</td>
<td>Cephalothin</td>
<td>Extensive consolidation 75 percent left upper, 90 percent right upper lobe. Variegated reddish-gray pattern on cut surface.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>54/M</td>
<td>4</td>
<td>Pulmonary Edema Pneumonia</td>
<td>Folic Acid deficiency Anemia Pylonephritis</td>
<td>Penicillin</td>
<td>Bilateral straw-colored pleural effusion (400cc). Edema and congestion - all lobes. Consolidation right lower lobe.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>66/F</td>
<td>14</td>
<td>Pulmonary edema with hemorrhage Pneumonia</td>
<td>ASCVD* Angina Pectoris</td>
<td>Cefotaxim</td>
<td>Petechial hemorrhage pleural surface. Bilateral hemorrhagic pleural effusion (500 cc). Diffuse severe edema and patchy pneumonia all lobes.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>49/M</td>
<td>7</td>
<td>Bilateral Pneumonia</td>
<td>Congestive Heart Failure</td>
<td>Cefotaxim</td>
<td>Diffuse bilateral pneumonia, edema and congestion all lobes.</td>
<td></td>
</tr>
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</table>

Arteriosclerotic cardiovascular disease
Pennsylvania State Department of Health, were over 40 years of age, had significant underlying disease and expired between the fourth and 14th day after the clinical illness was noted. The immediate cause of death was ascribed to respiratory failure (4 cases) and cardiac failure (1 case).

Formalin-fixed, paraffin embedded tissue specimens from lung (5 cases), liver and spleen (4 cases) were stained by the haematoxylin and eosin, periodic acid-Schiff, Gridley's reticulin, Verhoeff-Van Gieson, Perls' prussian blue and phosphotungstic acid-hematoxylin methods. To compare staining characteristics of microbial agents, sequential sections of lung tissue specimens were stained by the Brown-Brenn, Giemsa, Dieterle silver-impregnation methods and direct fluorescent antibody (FA) technique. Lung tissues in cases 1 and 5 were also examined with an electron microscope. Finally, haematoxylin and eosin and Giemsa stained lung tissue slides from fatal cases of human psittacosis infected with the Epizootic Bovine Abortion1 and Borg2 strains of *Chlamydia psittaci* were compared directly to LD victim specimens. Control tissue for the Dieterle stain and specific conjugate for fluorescent staining was kindly supplied by the CDC.

Results

The histopathology of lung tissue was similar in all five cases regardless of the day of illness prior to death. The inflammatory response was almost totally limited to the lower respiratory tract and involved respiratory bronchioles, alveolar ducts and alveoli. The bronchial branches often had light chronic inflammatory infiltrates within their walls, consisting mainly of lymphocytes, but destruction of the mucosa was rare or absent even in those with luminal cellular exudates. There was considerable variation in the extent and nature of the consolidation, often within the same lobule. Two distinctive patterns of involvement were recognized. Dense consolidation, extending into uninvolved area by direct alveolar spread, was most commonly seen (figure 1).

In other sections, dilated alveolar ducts, some filled with exudates, were found scattered throughout normal appearing lung (figure 2). Septal and interstitial areas were congested with red blood cells but contained few inflammatory cells. Those alveoli located at the periphery of the inflammatory reaction contained fewer numbers of inflammatory cells; however, the components of the exudative response were similar to those found in central dense areas of inflammation. Hypertrophied alveolar lining cells, many of which were desquamating in cohesive sheets, were frequently found at the margin of the inflammatory response (figure 3). The contents of the alveolar exudate commonly varied from field to field. Most involved alveoli contained a fibrinocellular exudate composed of large, occasionally vaculated mononuclear cells, red blood cells, polymorphonuclear cells, lymphocytes and alveolar epithelial cells in various proportions (figure 4). Erythrophagocytosis and leukophagocytosis by macrophages was frequently encountered (figure 5). The polymorphonuclear leucocyte content varied, but they were in the main fewer in number than the mononuclear cells. Fibrin, where present, was in the form of intra-alveolar meshwork with entrapped cells, not splayed on the septa as a hyaline membrane (figure 5). Interstitial fibrosis, or organizing obliterator alveolitis, was not seen. Pulmonary arterial and venous thrombi or emboli were absent.

Hepatic and splenic tissue showed marked acute congestion. There was moderate hepatic steatosis and moderate Kupffer cell hypertrophy. Occasional hepatic Kupffer cells and macrophages in the splenic red pulp showed erythrophagocytic and leucocytic phagocytosis. There were small numbers of immunoblasts and ma-
Figure 1. (Top) Margin of consolidated alveoli and normal lung. Note the lack of inflammatory response in the small bronchus (arrowhead). Hematoxylin and eosin stain, magnification $\times 125$.

Figure 2. (Bottom) Scattered area of intra-alveolar exudates of varying densities, not associated with dense consolidation. Hematoxylin and eosin stain, magnification $\times 125$. 
ture plasmocytes in the red pulp. The malpighian corpuscles were not enlarged and showed no active germinal centers.

Bacteria were not found in LD lung tissue stained by the methods used to demonstrate microorganisms including the Dieterle silver-impregnation method and direct fluorescent antibody staining.

**Comments**

Histopathology of lung tissue from these five cases of LD depict features commonly described as diffuse alveolar damage. The response of the lung to alveolar damage is similar, regardless of the causative agent or mechanism, and is manifest by injury to alveolar lining cells, which leads to fluid and cellular exudation. Numerous and similar agents have been reported to insite this inflammatory tissue response including: nickel carbonyl; oxidant gases \((\text{N}_2\text{O}, \text{O}_2, \text{O}_3)\); vapors of mercury, phosgene and chlorine; chemicals such as kerosene and paraquat;
Figure 5. High powered view showing fibrinocellular, mononuclear exudate, and erythro-leukophagocytosis by macrophages (arrowhead). Geimsa stain, magnification x 1000.

and a group of clinical diseases associated with the adult respiratory distress syndrome.\textsuperscript{11} Of the infectious agents known to cause this histopathologic response, only influenza and feline calicivirus have been described completely.\textsuperscript{12} Our cases were not exposed to any of these agents, except case 4 who received high concentrations of $O_2$ while receiving artificial ventilation.

A number of outbreaks of human chlamydial infections of the psittacine type\textsuperscript{17,19} as well as experimental disease in primates\textsuperscript{21} has been reported, which allows comparison to be made with the histopathology of these five cases of LD. A review of these published reports and direct comparison to lung tissue from two cases of psittacosis\textsuperscript{1,2} reveal the following similarities: (1) marked variability in the degree and characteristic of the intra-alveolar process, often within the same lobule; (2) mononuclear cell predominance; (3) sloughed hypertrophied alveolar lining cells, red blood cells, macrophages and mononuclear cells in varied proportions embedded in a fibrin meshwork; (4) virtual absence of significant interstitial inflammatory cellular exudate; (5) the presence of erythro-leukophagocytosis by macrophages; (6) little or no involvement of the larger bronchi or bronchioles; and (7) absence of clearly identifiable organisms with standard staining techniques.

Mononuclear cells and macrophages are the predominant cells found within alveoli during the resolution stage of common bacterial pneumonias, but are uncommonly found during the acute stage except in certain zoonoses owing to rickettsial or bacterial agents.\textsuperscript{24} Although the patchy consolidation and mononuclear intra-alveolar exudates are characteristic of rickettsial pneumonias, interstitial involvement, bronchial epithelial necrosis and perivascular cuffing usually described in this entity\textsuperscript{2,18} were not observed. The marked interstitial and alveolar septal infiltrates and the bronchial epithelial necrosis typically seen in viral pneumonias\textsuperscript{8} were also not found. Of the bacterial agents, tularemia, plague, anthrax and brucellosis are associated with mononuclear intra-alveolar exudates. However, areas of necrosis, vasculitis, bronchial inflammatory reaction and identifiable microorganisms with standard staining techniques are histologic features characteristically associated with these bacterial pathogens\textsuperscript{24} and were not observed in our cases of LD.

Inhalant toxins exert their effect in the respiratory tract depending upon their solubility in water. Vapors of hydrochloric acid, hydrofluoric acid, formaldehyde and
ammonia are highly soluble and affect the mucous membranes of the nose, throat and upper respiratory tract. In contrast, phosgene nitrous oxide, cadmium oxide, nickel carbonyl fumes and some halogenated hydrocarbons are less soluble and cause severe injury to the respiratory bronchioles and alveolar lining cells, while sparing the upper respiratory tract.7,25,26 Clinical symptoms produced by the less soluble agents are often delayed for hours to days.7,26 The histopathologic similarities between some toxic and infectious pneumonias were noted by Winternitz30 while studying the pathology of phosgene poisoning. At the same time, he called attention to the high incidence of secondary infection complicating injuries caused by inhalation of toxic gases.20

Diffuse alveolar damage is the active stage of an inflammatory response, the morphogenesis of which depends upon the nature of the inciting agent and duration of exposure. Resolution may occur without residual damage or progress to pulmonary interstitial or alveolar fibrosis, atelectasis or emphysema.11

Although the natural history of patients with LD pneumonia has not been determined, pulmonary function studies performed on LD survivors two years after the outbreak showed abnormalities in the diffusion capacity. Also, the clinical findings of shortness of breath and recurrent bronchitis suggest permanent lung damage may follow the resolution of Legionnaires' Disease pneumonia.16 Additional follow-up studies will be necessary to determine if long-term sequela develop.

Although our findings are similar to those reported by the CDC,3 we differ in regards to some histopathologic characteristics and in our failure to identify the organisms by special stains. Of 26 autopsied cases, the CDC reports finding an acute fibrinopurulent pneumonia in 21 cases and diffuse alveolar disease in 13 cases. The significance of the diffuse alveolar damage has not been explained,3 but its presence is often associated with lung injury owing to viral pneumonias, oxidant gases, chemical toxins, shock, pancreatitis and several drugs,11 and much less frequently with infection by bacteria.24 That bacteria were found by the Dieterle silver-impregnation stain in areas of classic fibrinopurulent pneumonia but not in areas of acute diffuse alveolar damage suggest the tissue action patterns may be due to separate agents. This combination of tissue reaction patterns has been described in cases of pneumonia secondary to influenza or toxic gases complicated by secondary infection owing to bacteria.30,31

Although highly sensitive, the Dieterle method cannot be used to identify specifically the LD bacteria because it stains other bacteria, chromatin, hemosiderin phagocytic granules, formalin pigment and melanin in a non-specific fashion.27 A method of direct fluorescent antibody (FA) staining of deparaffinized lung tissue sections has been described,6 which should allow for a more specific bacteriologic diagnosis in tissue sections. However, in a previous report, the CDC failed to identify the agent in 30 lung tissue specimens from five LD victims using pooled convalescent serum conjugated with fluorescein isothiocyanate (FITC) or radioactive iodine.20 Using FITC direct FA conjugate prepared from hyperimmune sera from goats or Legionnaires' Disease survivors, we failed to demonstrate organisms by the direct FA test in lung tissue sections or tissue scrapings from these five cases.

Also, in contrast to the CDC report, we found only the reaction pattern of diffuse alveolar damage with intra-alveolar mononuclear predominant cellular exudate, macrophages, and prominent erythrophagocytosis. There was a paucity of polymorphonuclear leukocytes in all five cases and bacteria were not found on special stains, including the Dieterle silver impregnation and direct fluorescent
antibody methods. Our lung tissue samples were taken from patients who expired at different time intervals during the acute illness and the exudative process was florid in all cases. Furthermore, we studied areas located at the periphery of the inflammatory reaction usually considered the area of most recent involvement, as well as the central dense areas of inflammation. Perhaps tissue specimens from these five cases were not from similar areas of lung involvement, when compared to the CDC's samples, as we found neither a fibrinopurulent reaction nor organisms.

Histopathologic findings in lung tissue of LD victims from other outbreaks or sporadic cases of Legionnaires' Disease vary considerably. Report findings include: exudative bronchopneumonia, ulcerative bronchitis with focal necrosis of bronchial epithelial cells, interstitial pneumonitis with edematous alveolar septa, infiltrated with polymorphonuclear leukocytes and macrophages; necrosis, microabscesses, interstitial fibrosis and coagulation necrosis. These findings were also not seen in these five cases from the Philadelphia epidemic.

It seems clear that some of the histopathologic findings in LD pneumonia may be due to secondary infection, the results of therapy or underlying disease. Final definition of the pulmonary histopathology must await studies in primates infected with aerosolized LD agent, as performed by McGavran when studying the histopathology of psittacosis.

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