Fluorescent-Antibody Detection of *Legionella dumoffii* in a Fatal Case of Pneumonia

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*Legionella dumoffii* organisms were detected in the lung tissue from a patient with a fatal case of pneumonia, the second reported to date. A fluorescent-antibody conjugate specific for the Tex-KL organism isolated in 1979 revealed many *L. dumoffii* organisms in lung tissue obtained postmortem from this patient.

The growing importance of legionellae as a pulmonary pathogen for man has been well documented. The bacterial strains designated as WIGA (2, 11) and MI-15 (GA-PH) (4, 15) have recently been classified as members of a new species of *Legionella, Legionella bozemanii* (1). The TATLOCK, HEBA (7), and “Pittsburgh pneumonia agent” strains (9, 14) belong to another species, *Legionella micdadei* (8) (for which the name *Legionella pittsburgensis* also has been proposed [13]). All of these strains share phenotypic similarities with *Legionella pneumophila* and cause disease in man, yet they are genetically distinct from *L. pneumophila* and are justifiably considered to be new species.

Recently, Lewallen and co-workers (10) reported the isolation and characterization of a legionella-like organism, Tex-KL. This organism was isolated from a postmortem lung specimen of a patient who died of pneumonia. The Tex-KL isolate is essentially identical to the NY-23 bacterial isolate obtained from a cooling tower (4) when compared by using gas-liquid chromatography analysis of the cellular fatty acids, deoxyribonucleic acid homology studies, and direct fluorescent-antibody (FA) staining. The name *Legionella dumoffii* has been proposed for this newly described bacterial species (1). We report here the occurrence of a second fatal case of pneumonia caused by *L. dumoffii* organisms in an immunosuppressed patient.

**CASE REPORT**

The patient was an 18-year-old female with systemic lupus erythematosus who was admitted to a hospital in San Antonio, Texas, on September 17, 1979, with a chief complaint of dyspnea and hemoptysis. She had been well until June 1979, when she visited her family physician with complaints of dyspnea, malaise, and tea-colored urine. Systemic lupus erythematosus was diagnosed in August 1979 after laboratory tests revealed a positive lupus erythematosus preparation and positive antinuclear antibody, and renal biopsy revealed a diffuse proliferative glomerulonephritis type III with numerous “wire loop” lesions.

She had previously been admitted on September 12, 1979, with hemoptysis and treated for pulmonary hemorrhage. Methylprednisolone sodium succinate, 60 mg daily, was started, and the patient was discharged on September 14, 1979.

At the time of her last admission, September 17, the patient was dyspneic with prominent hemoptysis. Physical examination revealed bilateral moist rales over the mid-lung fields, and chest radiographs revealed a new right lower lobe infiltrate in addition to bilateral alveolar infiltrates. Arterial PO₂ was 44 mmHg on room air. Methylprednisolone was increased to 1 g daily for 3 days, and cyclophosphamide (2 mg/kg per day) was started. After initial partial clearing of the pulmonary infiltrates, the patient’s condition deteriorated with progressive hypoxemia. Additional complications included a Coombs-positive hemolytic anemia, thrombocytopenia of 13,000/mm³, hypertension, and fever spikes to 103°F. Routine sputum, blood, and urine cultures revealed no pathogens other than a moderate growth of *Staphylococcus aureus* from the sputum culture. Antibiotic therapy, which included carbencillin, gentamicin, and sulfamethoxazole, was instituted. The patient was given 2 g of carbencillin every 12 h for 3 days before death and a total of 7 intravenous injections of gentamicin for 4 days before death in which blood levels of gentamicin were obtained. Two doses of sulfamethoxazole (20 mg each) were administered through a nasogastric tube 1 day before death.

The patient experienced persistent hypoxemia with bilateral pulmonary infiltrates and hemoptysis necessitating increased positive end expiratory pressure ventilation with 100% O₂. The patient progressively deteriorated and expired on hospital day 8 despite all measures. Permission for an autopsy was obtained.
MATERIALS AND METHODS

A portion of the lung obtained at autopsy was placed in 10% Formalin and was submitted to the Bureau of Laboratories, Centers for Disease Control. Lung smears were prepared by scraping the Formalin-fixed lung as described by Cherry et al. (3). The smears were heat fixed and stained with the FA conjugate for *L. pneumophila* serogroups 1 to 4 (16), serogroup 5 (5), serogroup 6 (12), and *L. bozemanii* (WIGA), *L. micdadei* (TATLOCK), and *L. dumoffii* (Tex-KL). Sections of the Formalin-fixed lung were embedded in paraffin, and the blocks of lung and other organs were cut 4 to 5 μm in thickness, deparaffinized, and stained with hematoxylin and eosin and the Dieterle silver impregnation stain (17).

The specificity of the *L. dumoffii* (Tex-KL) conjugate was tested by staining 250 miscellaneous cultures representing 11 genera and 24 species, including species of *Legionella, Pseudomonas, Flavobacterium, Acinetobacter, Haemophilus, and Streptococcus*. Smears of pure cultures were made from 1% Formalin-saline suspensions, heat fixed, and stained with the routine test dilution. Positive control smears were prepared with the Tex-KL human strain and the NY-23 environmental isolate.

RESULTS AND DISCUSSION

In addition to the manifestations of systemic lupus erythematosus and the associated type III diffuse proliferative glomerulonephritis, other clinicopathological findings at autopsy were mainly limited to the chest. The lungs weighed 2,700 g. Both lungs showed extreme congestion, edema, and consolidation with marked intraalveolar hemorrhage.

Microscopically, sections of the lungs showed diffuse intraalveolar hemorrhage, dense intraalveolar exudates containing scattered neutrophils and macrophages, and a few hyaline membranes. Lymphocytes and plasma cells were present in the interstitium. A Dieterle silver impregnation stain revealed numerous small intracellular and extracellular bacilli morphologically similar to *L. pneumophila*. Electron microscopy demonstrated organisms within the cytoplasm of pulmonary macrophages (Fig. 1). A Brown and Brenn stain revealed only a few bacilli; these were weakly gram positive.

Routine cultures of the autopsy lung tissue were done on sheep blood agar, Columbia agar with colistin, MacConkey agar, Trypticase soy broth (BBL Microbiology Systems), Schaedler broth, and a commercially prepared chocolate agar with 1% IsoVitaleX and 1% hemoglobin. Heavy growth of *Citrobacter freundii* was only observed on the chocolate agar plate. Swab cultures of the lung taken aseptically at autopsy and the postmortem blood yielded no growth after 72 h. Culturing was not attempted on charcoal-yeast extract agar (6), as it was not available.
at the time of autopsy. This agar is probably the best medium currently available for isolating *L. pneumophila*. This culture medium was used to isolate both the Tex-KL and the NY-23 strains of *L. dumoffii* and would have been used for attempted isolation in the present case, but unfixed tissue was not available at the time the organism was identified by direct FA staining at the Centers for Disease Control.

Lung scraping smears were FA negative with all conjugates of *L. pneumophila* serogroups 1 to 6, *L. bozemanii*, and *L. micdadei*. Smears and sections stained with the *L. dumoffii* (Tex-KL) conjugate revealed 25 to 30 brightly stained organisms per oil immersion field. The organisms visible in the Dieterle silver impregnation stain corresponded in location and morphology to the fluorescent bacteria observed in the lung section with the Tex-KL conjugate. No fluorescent bacteria were observed in the sections of liver, spleen, or kidney.

None of the 250 pure cultures tested with the routine test dilution of the Tex-KL conjugate stained at more than 2+ intensity. Only the positive controls, Tex-KL, and NY-23 bacteria stained with a 4+ intensity.

In all cases of pneumonia of unknown etiology, lung tissue should be taken aseptically and quick frozen for future studies, so that new strains of *Legionella* organisms may be isolated and characterized. Although no isolate was obtained, the demonstrated specificity of the Tex-KL conjugate indicates that the pneumonia in this immunocompromised patient was caused by *L. dumoffii*.

**LITERATURE CITED**


