Legionella micdadei (Pittsburgh Pneumonia Agent): Direct Fluorescent-Antibody Examination of Infected Human Lung Tissue and Characterization of Clinical Isolates

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Legionella micdadei (Pittsburgh pneumonia agent) was identified by direct fluorescent-antibody (DFA) examination of lung tissue in six of seven persons diagnosed previously as having *L. micdadei* pneumonia only by histopathology and in four persons who also had positive cultures of the organism. No cross-reactions occurred with monospecific DFA conjugates prepared against *Legionella pneumophila* serogroups 1 to 6, *Legionella bozemanii*, *Legionella dumoffii*, and *Legionella gormanii*. One person had *L. pneumophila* serogroup 6 identified by DFA examination of lung tissue and subsequent culture of stored pulmonary secretions. Characterization of the four strains of *L. micdadei* revealed specific DFA reactions, bacteriological behavior, and cellular fatty acid composition that allow identification of the organism. DFA testing appears to be a sensitive method for identifying *L. micdadei* present in human lung tissue or cultured on artificial media.

Legionella micdadei is a fastidious bacterium (8) which was originally isolated as the TAT-LOCK and HEBA strains (7) and subsequently shown to be an etiological agent of pneumonia (9, 14, 16). Of the eight cases of pneumonia originally described from Pittsburgh (14), five were presumptively identified solely by visualization of typical gram-negative, weakly acid-fast bacilli in lung tissue. Two other cases of *L. micdadei* pneumonia, one from Derby, Conn., and one from Boston, Mass. (1), also were similarly identified.

Recent cultivation of *L. micdadei* on artificial media such as charcoal-yeast extract (CYE) agar (9) and buffered charcoal-yeast extract (BCYE) agar (15) has resulted in further establishing its bacteriological similarity to *Legionella pneumophila* and other *Legionellaceae*, such as *Legionella bozemanii* (formerly called WIGA), *Legionella dumoffii* (formerly called Tex-KL and NY-23), and *Legionella gormanii* (formerly called LS-13) (3, 7, 9, 10, 15, 19). Agar cultivation has also aided in the development of a direct fluorescent-antibody (DFA) test that is apparently specific for *L. micdadei* (7, 9). Using this DFA test, the lung tissue of each of the seven presumptively identified cases of *L. micdadei* pneumonia was examined as were those of four

patients with *L. micdadei* pneumonia (including two recent unpublished cases) from which the organism was isolated either by inoculation of embryonated eggs or BCYE agar. The four strains of *L. micdadei* were also characterized.

MATERIALS AND METHODS

Tissue specimens from the previously reported patients with *L. micdadei* pneumonia were either paraffin-embedded sections of lung tissue or wet Formalin-fixed sections. Material examined from two recent unpublished cases that occurred in Pittsburgh consisted of homogenates of lung tissue taken during lung biopsy. DFA staining was performed as previously described (2-4, 9, 11, 12), using monospecific conjugates prepared against *L. micdadei*, *L. pneumophila* serogroups 1 to 6, *L. bozemanii*, *L. dumoffii*, and *L. gormanii*.

The four strains of *L. micdadei* were all initially isolated by culture of lung tissue in embryonated eggs (15). Subsequently, the bacteria were recovered from infected yolk sac membranes by diluting the yolk 1:1,000 in sterile, distilled water and inoculating 0.1-ml volumes of the diluted yolk onto BCYE agar as described by Pasculle et al. (15). The isolates were then tested for growth on CYE, Feeley-Gorman, and sheep blood agars (BBL Microbiology Systems, Cockeysville, Md.) as previously described (5, 6, 15). The identity of the isolates was confirmed by DFA staining (2, 7, 9) and gas-liquid chromatographic analysis of 3 to 5 day

old CYE agar-grown isolates (2-7, 9, 11-13) and failure to grow on Feeley-Gorman and sheep blood agars. Biochemical testing of the isolates was performed using the API 20E system (Analytab Products, Plainview, N.Y.). Testing for beta-lactamase activity was performed by the method of Thornsberry and Kirven (20).

RESULTS

Of the 11 tissues, 10 had *L. micdadei* identified by DFA examination. Numerous fluorescent organisms were seen both intra- and extracellularly in tissues stained with the *L. micdadei* conjugate. No staining of organisms occurred with DFA conjugates prepared against other *Legionellaceae*. The remaining case, one of the original five in the report of Myerowitz et al. (14), had *L. pneumophila* serogroup 6 detected by DFA examination. This diagnosis was subsequently confirmed by recovery of *L. pneumophila* serogroup 6 from a tracheal aspirate from this patient which had been stored at -70°C for 2.5 years.

The four strains of *L. micdadei* isolated from these cases were identical to each other and to the strain originally examined by Pasculle et al. (15) and Hébert et al. (9). All isolates grew well on CYE or BCYE agar, grew poorly or not at all on Feeley-Gorman agar, and failed to grow on sheep blood agar. They were catalase, oxidase, and gelatinase positive and did not produce beta-lactamase. Gas-liquid chromatographic analysis of the strains revealed a fatty acid profile similar to that previously described for this organism (7, 9, 15), with the most abundant fatty acid being a 15-carbon, branched-chain fatty acid (a15:0). The isolates all stained with the *L. micdadei* DFA conjugate and failed to react with conjugates prepared against other *Legionellaceae*. Although *L. micdadei* is weakly acid fast in infected human lung tissue, all of the isolates grown on agar were not acid fast with standard or modified (1% H_2SO_4) Ziehl-Neelsen stains. In contrast, the *L. pneumophila* serogroup 6 isolate grew well on Feeley-Gorman agar, was catalase positive, and had a 16-carbon, branched-chain fatty acid (a16:00) as its most abundant fatty acid.

DISCUSSION

This report documents the identification of *L. micdadei* by DFA staining in lung tissue from six of seven presumptively identified cases of *L. micdadei* pneumonia as well as four of four cases of bacteriologically confirmed disease. These data suggest that DFA examination is at least as sensitive as histopathology in detecting *L. micdadei* in lung tissue and is probably more

specific. The lack of heterologous DFA reactions with monospecific conjugates prepared against other species of *Legionellaceae* is reassuring.

The results of this study are consistent with those of Thomason et al. (18), who identified *L. micdadei* by DFA examination of the lung tissue of five patients in Virginia presumptively identified as having *L. micdadei* pneumonia by histopathology (17). They also found no cross-reactions with DFA conjugates prepared against other *Legionellaceae*. Hébert and colleagues (9) tested the *L. micdadei* DFA conjugate against 138 isolates representing 22 species of eight genera and found no positive reactions. These investigators also tested the conjugate against 82 *L. pneumophila* isolates and other *Legionellaceae* and found only flagellar staining. Whether the cross-reactivity of flagellar antigens among the *Legionellaceae* will pose a problem in the future, remains to be seen.

The description of four additional clinical isolates of *L. micdadei* confirms the characteristics previously reported (7, 9, 15). Each isolate had a strong homologous DFA reaction to *L. micdadei* and had by gas-liquid chromatographic analysis a characteristic fatty acid profile with predominance of the a15:0 branched-chain fatty acid. Biochemical reactions were consistent with previous reports in which the organism was described as catalase, oxidase, and gelatinase positive and without beta-lactamase activity (7, 9, 15).

One of the seven presumptively identified patients was found to have infection by *L. pneumophila* serogroup 6 by both DFA examination and culture of stored respiratory secretions. This case demonstrates that the tissue acid-fast staining characteristic may not be unique to *L. micdadei* and may be shared with at least some other species of *Legionellaceae*. Preliminary evidence indicates that *L. bozemanii* may also be weakly acid fast in tissue (Myerowitz and Pasculle, unpublished data). *L. micdadei* strains, however, lose their acid fastness when cultivated on agar. At present, we have no explanation for these unusual staining properties.

DFA testing thus appears to be of value in the identification of *L. micdadei* in human tissue or cultures. It is probable that DFA testing of other specimens such as pleural fluid or respiratory secretions will be of value for the diagnosis of *L. micdadei*, but the diagnostic sensitivity and specificity of this method need to be defined. Since the completion of the work described in this report, we have successfully detected *L. micdadei* in a transtracheal aspirate of another patient by DFA testing and confirmed the di-

agnosis by culture of the specimen on BCYE agar (Pasculle, manuscript in preparation).

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