Seroreactivity to *Mycoplasma pneumoniae* and *Legionella pneumophila*: Lack of a Statistically Significant Relationship

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We reviewed antibody titers to *Mycoplasma pneumoniae* and *Legionella pneumophila* serogroup I in sera from 1,060 cases of acute respiratory infection to determine whether there was an association in seroreactivity to these organisms. Of the 170 serum pairs with antibodies to *L. pneumophila* (35 seroconversions and 135 with presumptive titers), 32 (18.8%) demonstrated seroreactivity to *M. pneumoniae* (17 seroconversions and 15 with presumptive titers). This frequency was not significantly greater than the seroreactivity to *M. pneumoniae* observed in sera without antibodies to *L. pneumophila* (17.5%) (0.05 < P < 0.10), which included 111 seroconversions and 45 sera with presumptive titers.

Laboratory confirmation of Legionnaires disease is usually required because clinical differentiation between pneumonias induced by *Legionella pneumophila* and other established agents is difficult. The most frequently used laboratory test for confirming a diagnosis of Legionnaires disease pneumonia is the detection of increasing antibody levels by the indirect immunofluorescence test (14). Because of the reliance on this retrospective serological test, the association between *Mycoplasma pneumoniae* seroreactivity and *L. pneumophila* infections reported by Grady and Gilfillan (2) is of particular concern. These authors reported that significant titers of complement-fixing antibody to *M. pneumoniae* occurred in 22 of 27 cases of serologically diagnosed Legionnaires disease.

To assess the frequency and importance of serological coreactivity between *L. pneumophila* and *M. pneumoniae*, we reviewed the reactivities of the sera from 1,060 cases of acute respiratory tract infections to both antigens.

**MATERIALS AND METHODS**

**Sera.** Acute and convalescent sera from 1,060 patients with acute respiratory infections were selected for this study. These sera had been submitted to the University Hygienic Laboratory between 1971 and 1978 for diagnostic serological tests. The sera received between 1971 and 1977 were assayed on receipt for antibodies to *M. pneumoniae* and were then stored at −25°C in tightly sealed, screw-capped vials until they were tested for antibodies to *L. pneumophila* in 1978. Sera submitted during 1978 were assayed on receipt for antibodies to *M. pneumoniae* and *L. pneumophila*. Randomly selected sera examined in 1972 and 1973 for complement fixation antibodies to mycoplasmal antigens were reexamined in 1978. The titers obtained in 1972 and 1973 were not significantly different from those obtained in 1978.

**Antibody assays.** Antibodies to *L. pneumophila* were assayed by the immunofluorescence antibody test, using the method of McDade et al. (9) and either diethyl ether-killed or heat-killed *L. pneumophila* serogroup I (Philadelphia I) as the antigen. The antigens obtained by these two methods yielded comparable titers. The reagents for this test were supplied by the Centers for Disease Control, Atlanta, Ga. We used a standard complement fixation assay to measure antibodies to the lipid antigen of *M. pneumoniae* (13).

For the purpose of our analysis, seroconversions were defined as sera with titers that increased at least fourfold between the acute and convalescent phases of illness to levels of at least 1:32 for *M. pneumoniae* and 1:128 for *L. pneumophila*. Sera with presumptive antibody titers were defined as those serum pairs in which the standing titer of at least one serum of the pair was 1:32 or more for *M. pneumoniae* or 1:256 or more for *L. pneumophila.**

**RESULTS**

Table 1 shows the frequency of *M. pneumoniae* titers in sera with and without elevated titers against *L. pneumophila* serogroup I. Of the 35 serum pairs with seroconversions to *L. pneumophila*, 3 (8.6%) also demonstrated seroconversions to *M. pneumoniae*, and 14 (10.4%) of 135 sera with presumptive titers to *L. pneumophila* had seroconversions to *M. pneumoniae*. There were an additional 111 (12.5%) seroconversions to *M. pneumoniae* among 890 paired...
TABLE 1. Frequency of antibodies to M. pneumoniae in L. pneumophila-positive sera

<table>
<thead>
<tr>
<th>L. pneumophila serogroup I seroreactivity of serum pairs</th>
<th>No. with complement-fixing antibodies against M. pneumoniae showing:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Seroreactions</td>
<td>Total no.</td>
</tr>
<tr>
<td>Seroreactions</td>
<td>35</td>
</tr>
<tr>
<td>Presumptive titers</td>
<td>135</td>
</tr>
<tr>
<td>Negative</td>
<td>890</td>
</tr>
</tbody>
</table>

* Seroreactivities were determined by the indirect immunofluorescence test.

† Fourfold increase in antibody titer of ≥1:32.

‡ A single or standing titer of ≥1:32.

§ Fourfold increase in antibody titer of ≥1:128.

A single or standing titer of ≥1:256.

sera from patients with acute respiratory illness without elevated antibody titers to L. pneumophila.

When the percentages of seroconversions to M. pneumoniae found in sera with concomitant seroconversions (8.6%) or elevated titers (10.4%) to L. pneumophila were compared independently with the percentage of seroconversions (12.5%) in sera without titers to L. pneumophila, the ratios were not statistically significant (G test for independence; 0.05 < P < 0.10).

Presumptive titers to M. pneumoniae occurred in 15 (11%) of the 135 presumptive L. pneumophila sera and in 45 (5%) of the 890 negative L. pneumophila sera. None of the 35 sera pairs that had fourfold increases in antibody titers to L. pneumophila had elevated titers to M. pneumoniae. These differences were not statistically significant (0.05 < P < 0.10).

To confirm and expand these data, we determined the frequencies of L. pneumophila seroreactivity in sera positive for M. pneumoniae and in sera not positive for M. pneumoniae (Table 2). Of the 188 serum pairs (including 128 seroconversions and 60 with presumptive titers) with seroreactivity to M. pneumoniae, 32 (17.0%) had concomitant antibody titers to L. pneumophila (including 3 seroconversions and 29 with presumptive titers). This frequency was not significantly greater than the 138 (15.8%) serum pairs with seroreactivity to L. pneumophila which occurred among the 872 serum pairs without antibodies to M. pneumoniae. These serum pairs included 32 (3.7%) seroconversions and 106 (12.2%) with presumptive titers to L. pneumophila.

TABLE 2. Frequency of antibodies to L. pneumophila serogroup I in M. pneumoniae-positive sera

<table>
<thead>
<tr>
<th>M. pneumoniae seroreactivity of serum pairs</th>
<th>No. with antibodies against L. pneumophila serogroup I showing:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Seroreactions</td>
<td>128</td>
</tr>
<tr>
<td>Presumptive titers</td>
<td>60</td>
</tr>
<tr>
<td>Negative</td>
<td>872</td>
</tr>
</tbody>
</table>

* Seroreactivities were determined by the complement fixation test.

† Fourfold increase in antibody titer of ≥1:128.

‡ A single or standing titer of ≥1:256.

§ Fourfold increase in antibody titer of ≥1:32.

A single or standing titer of ≥1:32.

discerning disease. Unlike classic bacterial pneumonias, L. pneumophila and M. pneumoniae infections are seldom confirmed in the laboratory by isolation of the organism, but are most commonly confirmed by retrospective analyses of paired serum specimens. Therefore, if the sera from patients with legionellosis also contain antibodies to M. pneumoniae, as reported by Grady and Gilfillan (2), serological tests may not distinguish between these clinically similar diseases.

The results of this study indicate that there is no statistically significant increased frequency of titers to M. pneumoniae in sera with antibodies to L. pneumophila serogroup I. Furthermore, we could not demonstrate the converse situation (i.e., a significantly greater frequency of L. pneumophila seroreactivity in sera from patients with antibody titers to M. pneumoniae).

The difference between our results and those of Grady and Gilfillan may be explained by the method used to measure complement-fixing antibodies to Mycoplasma. Grady and Gilfillan used whole-cell M. pneumoniae antigen, whereas in this study we used lipid antigen extracted by chloroform-methanol from broth-grown M. pneumoniae. Lipid antigen is less anti-complementary, and greater differences in titer are found between acute and convalescent sera (4). Differences in case selection, high incidences of simultaneous infections in the Massachusetts patients, and differences in the antigenicities of infecting strains of organisms are alternate explanations that may account for the discordant findings.

Our results agree with those of Wilkinson et al. (15), who recently reported that among 50 epidemic M. pneumoniae sera, 7 (14%) (1 seroconversion and 6 with presumptive titers) had seroreactivity to L. pneumophila. Our results also agree with those of Taylor et al. (11), who reported that the serological diagnosis of the two
forms of pneumonia in the United Kingdom is usually not complicated. However, our results do not agree with their conclusion that sera containing antibodies to both of these agents are uncommon. It should be noted that Taylor et al. used Formalin-killed antigen, whereas we used both heat-killed and ether-killed L. pneumophila.

Approximately 19% of the 170 serum pairs with significant titers to L. pneumophila had seroreactivity to M. pneumoniae, and, conversely, 17% of the 188 serum pairs with antibody titers to M. pneumoniae had seroreactivity to L. pneumophila. Whether the observed copositivity in this study is related to antigen similarities of certain strains of M. pneumoniae and L. pneumophila or to simultaneous or sequential infections cannot be determined with certainty from the data available. However, since the observed incidence of copositivity was not greater than that expected from each infection alone, it is likely that infections by M. pneumoniae and L. pneumophila (or serologically related organisms) occurred in these patients. Coinfections with L. pneumophila have been reported for Acinetobacter sp. (8), Klebsiella pneumoniae (5), and M. pneumoniae (10). Wilkinson et al. were able to reduce the titer to L. pneumophila in two of seven copositive sera by absorption with an immunosorbent extracted from Escherichia coli O13:K92:H4, which suggests that some copositivity is nonspecific.

Although it is clear that antibodies with multiple specificities occur in the sera of survivors of Legionnaires disease (6, 7, 12, 16, 17), our findings indicate that demonstration of seroconversion by the complement fixation test for M. pneumoniae and the immunofluorescence antibody test for L. pneumophila can be used to differentiate these infections. Because of the frequency of each infection and the concurrent antibodies, presumptive titers are of less diagnostic value.

LITERATURE CITED