Serum Bactericidal Activity against *Legionella pneumophila*

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Two strains of *Legionella pneumophila* serogroup 1 (UH1 and RH1) were incubated in fresh human serum. The UH1 strain was serum resistant, whereas the RH1 strain was serum susceptible. The bactericidal activity of fresh serum was abrogated by heating. Serum resistance of *L. pneumophila* strains may correlate with increased virulence.

*Legionella pneumophila* has been found in many natural and man-made aquatic environments not associated with the presence of Legionnaires disease (2, 3). Subtypes of *L. pneumophila* have been defined by monoclonal antibody patterns and plasmid content (4, 5). Differences in the virulence of these subtypes may account for the disparity between environmental contamination and lack of disease. Two subtypes of *L. pneumophila* serogroup 1 (UH1 and RH1) are present in our medical center and have been associated with different attack rates (1.03 per 1,000 discharges for UH1 and 0.08 per 1,000 discharges for RH1) (7). The two subtypes also had different 50% lethal doses (7.4 × 10⁶ for UH1 and 9.1 × 10⁷ for RH1) in the guinea pig intraperitoneal infection model (1). Since resistance to the bactericidal activity of nonimmune human serum has been related to virulence in other gram-negative bacilli, we investigated this phenomenon with the two subtypes of *L. pneumophila* serogroup 1 prevalent in our hospital environment. *L. pneumophila* was isolated from potable water on buffered charcoal-yeast extract agar, passaged once, and frozen at −70°C until used. The stocks were later thawed, grown on buffered charcoal-yeast extract agar, washed off the plate, and diluted in water. Monoclonal typing and plasmid analysis were performed as previously described (4, 5).

Serum was obtained from eight volunteers and used within 1 h. Aliquots of heat-inactivated sera were prepared by heating to 56°C for 30 min. A pool of serum was obtained from 10 additional volunteers and frozen at −70°C. Antibody to *L. pneumophila* was determined by an indirect immunofluorescence assay with the two Formalin-fixed subtypes as antigens (6).

In the first set of experiments, 0.1 ml of *L. pneumophila* (UH1 or RH1) (approximately 10⁶ CFU) was added to 0.9 ml of fresh or heat-inactivated serum from the eight individual donors. Aliquots (0.1 ml) were serially diluted and cultured in triplicate on buffered charcoal-yeast extract agar after 0, 1, 6, and 24 h of incubation at 37°C. Table 1 shows the marked differences in the survival of the UH1 strain and the RH1 strain when they were incubated in 90% fresh human serum. The means of the groups were analyzed by the two-tailed Student *t* test. There was a 1-log decrease in the CFU of the UH1 strain and a 5-log decrease in the CFU of the RH1 strain over 24 h. When the sera were heated, the bactericidal activity was abrogated. Three of the donors had reciprocal indirect immunofluorescence assay titers for *L. pneumophila* of 64, 128, and 256. The other five had titers of <16. There was no difference in the bactericidal activity in the presence or absence of antibody.

In the second set of experiments, 10⁵ CFU of *L. pneumophila* (UH1 or RH1) in water was incubated for 6 h in fresh or heat-inactivated serum at final concentrations of 0, 10, 25, 50, and 90% in water. The CFU of the RH1 strain decreased 1 log in 10% serum and 4 logs in 25, 50, and 90% serum.

There was no bactericidal activity in heated serum. There was no decrease in the CFU of the UH1 strain at the various concentrations of either fresh or heat-inactivated serum. The reciprocal indirect immunofluorescence assay titer of the pooled serum was <16 for both strains.

Organisms which had been incubated in serum for 1 h were stained with fluorescein-labeled anticomplement antibody (Kallestad Laboratories, Chaska, Minn.). The RH1 strain stained more intensely than did the UH1 strain. No staining was observed when the organisms were incubated in the heated serum.

Serum resistance is a marker of virulence in other gram-negative bacilli (8). Serum resistance of the UH1 strain

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**TABLE 1. Survival of *L. pneumophila* in human serum**

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Fresh serum</th>
<th>Heated serum</th>
<th><em>P</em></th>
<th>Fresh serum</th>
<th>Heated serum</th>
<th><em>P</em> for UH1 in fresh serum vs RH1 in fresh serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UH1</td>
<td></td>
<td></td>
<td>RH1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.1 ± 0.5</td>
<td>6.4 ± 0.1</td>
<td>NS*</td>
<td>6.4 ± 0.1</td>
<td>6.3 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>1</td>
<td>6.1 ± 0.6</td>
<td>6.5 ± 0.1</td>
<td>NS</td>
<td>6.4 ± 0.4</td>
<td>6.5 ± 0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>5.7 ± 0.6</td>
<td>6.3 ± 0.2</td>
<td>0.04</td>
<td>3.6 ± 2.4</td>
<td>6.1 ± 0.4</td>
<td>0.03</td>
</tr>
<tr>
<td>24</td>
<td>5.1 ± 1.0</td>
<td>6.2 ± 0.2</td>
<td>NS</td>
<td>1.4 ± 2.1</td>
<td>6.2 ± 0.2</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

* Reported as the mean log CFU ± standard deviation for eight serum samples.

* NS, Not significant.

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correlates with the increased virulence of this strain in the guinea pig intraperitoneal infection model (1) and the more frequent isolation of this strain from patients with Legionnaires disease (7). The different major surface antigens recognized by monoclonal antibodies appear to be endotoxins (M. F. Para, W. E. Maher, and J. F. Plouffe, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 156, 1984). The different endotoxins may vary in their ability to bind complement or may affect the ability of complement to initiate lysis. Differences in the surface antigens of *L. pneumophila* subtypes may be related to virulence.

**LITERATURE CITED**


