Serological Differences in *Legionella pneumophila* Infections

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Guinea pigs were infected with two subtypes of *Legionella pneumophila* serogroup 1 (UH1 and RH1). Seroconversion by indirect fluorescent-antibody assay was demonstrated in 94 to 97% of guinea pigs when the challenge strain was used as the antigen. The standard Philadelphia 1 antigen demonstrated seroconversion in 94% UH1-challenged animals, but in only 66% of RH1-challenged animals.

Legionnaires disease is a bacterial pneumonia caused by *Legionella pneumophila* (3, 8). The organism can be difficult to culture (6), and the diagnosis is frequently made by seroconversion (17). The serological test that is most commonly employed is the indirect fluorescent-antibody (IFA) method of Wilkinson et al. (15-18). There are eight serogroups of *L. pneumophila* (2, 15). The serotyped antigens for the IFA test are usually pooled so that several serogroups can be screened at the same time (15). We have identified several subtypes of *L. pneumophila* serogroup 1 from the potable water system of our hospital (13). Two of these strains were associated with different buildings and different attack rates of nosocomial Legionnaires disease. We used the guinea pig intraperitoneal (i.p.) injection model of Legionnaires disease described by Fraser (4) to study the serological responses to infection produced by these two strains. The strains were characterized by monoclonal antibody reactivity (12) and plasmid content (7) as UH1 and RH1. The organisms were isolated on buffered charcoal yeast extract agar. Individual colonies were picked and streaked for lawn growth. The organisms were washed off the plates and frozen at −70°C in 50% Trypticase soy broth (BBL Microbiology Systems) and 50% glycerol until use. Before each experiment, the organisms were thawed, washed, and diluted to the desired concentration. Male Hartley guinea pigs (250 to 300 g) were infected i.p. with 1-ml suspensions of serial dilutions (10⁶ to 10⁰) of the two *L. pneumophila* strains. Guinea pigs had blood drawn by cardiac puncture on day 0 and day 30 for serological studies. Four groups of three guinea pigs received i.p. injections with either UV-killed or heat-killed UH1 or RH1 cells (10⁶).

The IFA assay was performed as described by Wilkinson et al. (17), except that Formalin-killed UH1, RH1, and Bellingham 1 strains were used in addition to the standard serogroup 1 heat-killed (Philadelphia 1) strain supplied by the Bureau of Biologics, Centers for Disease Control (CDC), Atlanta, Ga. All strains were acetone-fixed to the slides. Fluorescein-labeled goat anti-guinea pig immunoglobulin G was used in a working dilution of 1:32 (Cappel Laboratories, West Chester, Pa.).

Since the serological responses did not differ between the guinea pigs infected with live organisms and those injected with killed organisms, these results were analyzed together. Linear regression analysis was performed to determine the correlation of the reciprocal antibody titers to the various antigens. Student’s *t* test was performed to determine whether there were differences between the geometric mean reciprocal titers of the different antigen groups.

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Guinea pigs injected i.p. with UH1 and RH1 had differences in their serological responses (Table 1). When the challenge strain was used as the antigen, sereoversion was seen in 94 and 97% of the guinea pigs, respectively. When the serogroup 1 type strain (Philadelphia 1) was used as the antigen, sereoversion was seen in 94% of the UH1-challenged animals but in only 66% of the RH1-challenged guinea pigs. When the Bellingham strain was used as the antigen, only 14% of the UH1-challenged animals sereoverted, compared with 94% of the RH1-challenged animals. We conclude that some L. pneumophila serogroup 1 strains may not induce antibodies in guinea pigs which will be detected by the standard Philadelphia 1 strain. Further studies with convalescent sera from patients with Legioni-naires disease caused by the RH1 or Bellingham strain need to be performed to determine whether the Bellingham 1 strain should be added as an additional antigenic reagent for the IFA assay.

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LITERATURE CITED


### TABLE 1. IFA responses to two subtypes of L. pneumophila serogroup 1

<table>
<thead>
<tr>
<th>IFA antigens</th>
<th>UH1</th>
<th>RH1</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. of guinea pigs</td>
<td>Titer</td>
</tr>
<tr>
<td>Phil 1</td>
<td>34</td>
<td>153</td>
</tr>
<tr>
<td>UH1</td>
<td>34</td>
<td>107</td>
</tr>
<tr>
<td>RH1</td>
<td>34</td>
<td>10</td>
</tr>
<tr>
<td>Bell</td>
<td>14</td>
<td>9</td>
</tr>
</tbody>
</table>

* Two groups of guinea pigs were injected i.p. with the UH1 or RH1 strain of L. pneumophila serogroup 1. IFA titers were measured by using four serogroup 1 strains (CDC supplied Philadelphia 1 [Phil 1], UH1, RH1, and Bellingham 1 [Bell] strains). The results are reported as geometric means of the reciprocal titers and the percentage of sera showing a fourfold rise. All initial titers were <16.