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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 serotypic 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 (10^6 to 10^9) of the two live *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 (10^9).

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 antiguinea 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 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.

All preinjection sera had reciprocal titers of <16. Twenty-nine guinea pigs infected with UH1 survived and had serological studies. Six others received killed UH1 organisms. Most guinea pigs seroconverted (33 of 35) when the standard serogroup 1 antigen (Philadelphia 1) was used. The same 33 animals seroconverted when the UH1 antigen was used. The geometric mean reciprocal titer was 153 for the CDC antigen and 107 for the UH1 antigen, with a correlation coefficient of 0.80. These titer differences are consistent with reported (16) differences between heat-killed and Formalin-killed antigens. When the RH1 antigen was used in the IFA assay, 6 of 35 guinea pigs seroconverted with a mean reciprocal titer of 10 ($P < 0.001$). Two of 14 guinea pigs seroconverted when tested with the Bellingham antigen.

Only 21 of 32 (66%) guinea pigs injected with RH1 seroconverted when tested with the standard CDC serogroup 1 antigen, compared with 31 of 32 (97%) when the RH1 strain was used as the antigen. The mean reciprocal antibody titer with the CDC antigen, 41, was significantly lower than that with the RH1 antigen, 152 ($P < 0.001$). Correlation was low ($r = 0.27$). The mean titers obtained with the UH1 antigen, 13, were even lower than those with the CDC antigen ($P < 0.001$). The results obtained with the Bellingham strain correlated with those obtained with the RH1 strain ($r = 0.52$; $P < 0.001$).

L. pneumophila has been divided into at least eight serogroups on the basis of reactivity with rabbit antisera. The prototype strain for serogroup 1 has been the Philadelphia 1 strain, which has been used as the antigen in the IFA assay. Ormsbee et al. noted that one *L. pneumophila* serogroup 1 strain (LAW [11], Bellingham 1 strain [10]) had somewhat different surface antigen characteristics. Two guinea pigs infected with the Bellingham strain did not seroconvert when the Philadelphia 1 strain was used in the IFA assay. Benson et al. absorbed human convalescent sera with Philadelphia 1 and Bellingham 1 and concluded that the Bellingham strain contained immunogens only minimally present on Philadelphia 1 (1). Similar results were obtained by Thomason and Bibb (14) with absorbed rabbit antisera.

The development of monoclonal antibodies to *L. pneumophila* serogroup 1 has allowed further division of serogroup 1 strains into subtypes (5, 9, 12). The predominant clinical isolate from our patients is UH1, which is antigenically similar to Philadelphia 1. A second strain of *L. pneumophila* serogroup 1 isolated from our hospital environment and from one patient was classified as RH1 by monoclonal antibody reactivity and plasmid analysis (13). This strain had monoclonal reactivity similar to that of Bellingham 1 but, unlike Bellingham, contained two plasmids.

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TABLE 1. IFA responses to two subtypes of *L. pneumophila* serogroup 1[a]

IFA antigens	UH1			RH1		
	No. of guinea pigs	Titer	Convertors (%)	No. of guinea pigs	Titer	Convertors (%)
Phil 1	34	153	94	32	41	66
UH1	34	107	94	32	13	31
RH1	34	10	17	32	152	97
Bell	14	9	14	32	84	94

[a] 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.

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, seroconversion was seen in 94 and 97% of the guinea pigs, respectively. When the serogroup 1 type strain (Philadelphia 1) was used as the antigen, seroconversion 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 seroconverted, 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 Legionnaires 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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