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Cross-reactivity among *Legionella* species and serogroups

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SUMMARY

Cross-reactions of 17 members of the family Legionellaceae were studied by four different serological techniques: immunofluorescence (IF), slide agglutination (SA), microagglutination (MA) and immunodiffusion (ID), using antigens and rabbit antisera prepared in our laboratory. Results obtained corresponded closely with those described by other authors, especially for IF and SA.

The 17 antigens were further tested by IF with a panel of sera previously diagnosed as positive for legionella. A high number of positive reactions with several of the antigens tested were found, half of them being positive for *Legionella pneumophila* serogroup 1, usually in combination with other serogroups or species. The remaining sera presented a great variety of patterns combining different antigens.

INTRODUCTION

During the past few years a large number of species and serogroups of *Legionella* have been described (Wilkinson, 1987). As their recognition is basically made on serological grounds, the number of antigens and antisera necessary for the diagnosis of legionellosis has increased in parallel. The use of a large number of antigens and antisera from related organisms presents difficulties due to the appearance of cross-reactions. This is especially important with organisms belonging to the family Legionellaceae that have been reported to share a variety of antigens with bacteria from different levels of relatedness (Joly & Kenny, 1982; Collins *et al.* 1983*a*, *b*; Joly, Chen & Ramsay, 1983).

We studied the cross-reactivity of antigens and antisera prepared in our laboratory from 17 *Legionella* species and serogroups by four different serological techniques: slide agglutination (SA), microagglutination (MA), immunodiffusion (ID) and indirect immunofluorescence (IF).

In order to see if these cross-reactions appear in naturally acquired infections, sera from patients previously diagnosed as having legionellosis were titrated with our 17 antigens by IF.

MATERIALS AND METHODS

Strains were obtained from American Type Culture Collection and included: *L. pneumophila* serogroup 1 (Philadelphia 1); *L. pneumophila* serogroup 2 (Togus 1); *L. pneumophila* serogroup 3 (Bloomington 2); *L. pneumophila* serogroup 6 (Chicago 2); *L. pneumophila* serogroup 7 (Chicago 8); *L. pneumophila* serogroup 8 (Concord 3); *L. micdadei* (Tatlock); *L. bozemanii* (Wiga); *L. dumoffii* (NY-23); *L. gormanii* (LS-13); *L. longbeachae* serogroup 1 (Long Beach 4); *L. longbeachae serogroup* 2 (*Tucker* 1); *L. jordanis* (BL-540); *L. wadsworthii* (Wadsworth 81-716-A) and *L. oakridgensis* (OR-10). Strains Los Angeles 1 and Dallas IE formerly designated *L. pneumophila* serogroup 4 and 5 respectively and recently recognized as a new species by Selander *et al.* (1985), were also included.

Antigens. (*a*) Formalized antigens prepared as described by Wilkinson & Fikes (1980) were used for SA, MA and ID. (*b*) Heat-killed antigens prepared according to Wilkinson, Fikes & Cruce (1979) were used for IF.

Antisera. Obtained by immunizing rabbits with whole cells as described by Thacker, Wilkinson & Benson (1983). Antisera presenting any cross-reaction were absorbed with the involved strain.

Human sera. Thirty-eight sera positive to legionella were selected from our sera library.

Slide agglutination was performed according to Thacker, Wilkinson & Benson (1983).

Microagglutination. Performed as described elsewhere (Orgaz *et al.* 1986).

Immunodiffusion. Performed as described by Soriano, Aguilar & Garcés (1982).

Indirect immunofluorescence was performed as described by Wilkinson Fikes & Cruce (1979) for human sera, using FITC-labelled anti-rabbit gammaglobulin (Behring) as the conjugate, for the first part of the study, FITC-labelled anti-human gammaglobulin for the second. Titres above 64 were considered as positive.

RESULTS

Cross-reactivity tests

Table 1 shows the results of cross-reactions found by the four tests employed. All cross-reaction disappeared when antisera were absorbed with the corresponding strain.

Study of human sera

As shown in Table 2, only a small number of sera (10 of 38) gave a positive result with a single antigen. The remaining sera presented multiple positive reactions with up to eight different antigens.

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Table 1. *Cross-reactions between type strains and heterologous antisera*

Type strain	Agglutination	Microagglutination	Immunodiffusion	Immunofluorescence
L. pneumophila S1	*L. pn* 7	—	—	*L. pn* 7
L. pneumophila S2	*L. pn* 3	*L. jord*	—	*L. pn* 3, *L. pn* 6
L. pneumophila S3	Los Angeles 1	*L. pn* 2, *L. pn* 6	*L. pn* 2	*L. pn* 2, *L. pn* 6
Los Angeles 1	*L. pn* 8	—	Dallas 1E, *L. pn* 8	Dallas 1E, *L. pn* 8
Dallas 1E		—	*L. pn* 8	—
L. pneumophila S6	*L. pn* 2	—	*L. pn* 3	*L. pn* 3
L. pneumophila S7	*L. pn* 1	—	—	*L. dum*
L. pneumophila S8	*L. pn* 3, Los Angeles 1	Dallas 1E	Los Angeles 1	Los Angeles, Dallas 1E
L. micdadei		—	—	—
L. bozemanii	*L. long* 2, *L. jord*	*L. jord*	—	*L. jord*
L. dumoffii	*L. pn* 7	—	—	—
L. gormanii		—	—	—
L. longbeachae S1	*L. long* 2	*L. long* 2		*L. long* 2
L. longbeachae S2	*L. long* 1, *L. jord*	*L. long* 1, *L. jord*	*L. long* 1	*L. long* 1, *L. jord*
L. jordanis	*L. boz*	L. boz	—	*L. boz*
L. wadsworthii	*L. oakrid*	*L. oakrid*	—	—
L. oakridgensis		—	—	—

Table 2. *Results of 38 sera from patients with legionellosis studied by IF with the 17 legionella antigens prepared in our laboratory*

Number of positive sera	Antigens	Number of positive sera	Antigens
3	*L. pn* 1	1	*L. mic, L. gorm*
2	*L. pn* 2	2	*L. pn* 1, 6, 7
2	*L. pn* 3	1	*L. pn* 8, *L. boz*, Los Angeles 1
2	Los Angeles 1	1	*L. jord, L. long* 1, 2
1	*L. dum*	1	*L. pn* 2, 8, Los Angeles 1, Dallas 1E
3	*L. pn* 1, Los Angeles 1	1	*L. pn* 8, *L. dum*, Los Angeles 1, *L. boz*
2	*L. pn* 1, 6	1	*L. mic, L. boz, L. long* 1, 2
1	*L. pn* 1, 7	1	*L. pn* 1, 7, 8 *L. dum*, Los Angeles 1
1	*L. pn* 2, 3	1	*L. pn* 1, 6, 7, 8, *L. dum*
1	*L. pn* 3, 6	1	*L. pn* 1, 2, 3, 6, 7, 8, Los Angeles 1
1	*L. pn* 8, Dallas 1E	1	*L. pn* 1, 2, 3, 6, 7, 8, *L. dum*, Los Angeles 1
1	*L. pn* 6, 7	2	*L. pn* 1, 3, 6, 7, 8, *L. boz, L. dum*, Los Angeles 1
1	Los Angeles 1, *L. gorm*		

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DISCUSSION

In the study of cross-reactivity of type strains and antisera it is very remarkable that some of the crossings appeared with one method and not with the others; i.e. *L. longbeachae* serogroup 2 antigen and *L. bozemanii* antiserum presented a positive reaction by SA but not by IF. Moreover, sometimes a cross-reaction occurred in one direction when studied by one method and in the opposite direction by other method; i.e. *L. pneumophila* serogroup 7 antigen and *L. dumoffii* antisera by SA and the opposite by IF.

Most of the cross-reactions encountered by us have been reviewed by other authors (Thacker, Plikaytis & Wilkinson, 1985; Pelaz, Martin-Bourgon & Casal, 1984). However, the following crossings were found that have not been described previously: between *L. pneumophila* serogroup 1 and 7, between *L. pneumophila* serogroup 7 and *L. dumoffii* and between *L. oakridgensis* antigen and *L. wadsworthii* antiserum. In the study of sera from patients with legionellosis (Table 2), more than half of the sera were positive for *L. pneumophila* serogroup 1, a great diversity of multiple reactions being also encountered, but these were not the ones found with type strains and antisera. Similar findings were described by Wilkinson *et al.* (1983) when evaluating IF for antigens other than *L. pneumophila* serogroup 1. Fallon & Johnston (1987) have described the appearance of antibodies to several *L. pneumophila* serogroups or *Legionella* species in patients infected with *L. pneumophila* serogroup 1 in a common-source outbreak. In these, no constant pattern was found, suggesting that the response is a characteristic of the infected individual and not of the infecting strain of *Legionella* species. In another report, Fallon & Abraham (1983) demonstrated the presence of low-level antibodies to *L. longbeachae* serogroups 1 and 2 and *L. jordanis* without any apparent correlation with disease.

These discrepancies in results between the cross-sensitivity among type strains and rabbit antiserum against them and the results with human sera, must be stressed. The study of the serological response of patients with positive cultures for *Legionella* species other than *L. pneumophila* serogroup 1 would help to clarify the role of antibodies to these organisms.

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