Heterogeneity of antibody response in infection with *Legionella pneumophila* serogroup 1

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SUMMARY The antibody response of patients infected with *Legionella pneumophila* serogroup 1 in a common source outbreak was investigated. Heat-killed antigens from *L pneumophila* serogroups 1-3 and 6-10, plus several other strains of *L pneumophila*, together with 13 other species of legionellas were used in an indirect fluorescence antibody test. Formolised yolk sac antigens made from *L pneumophila* serogroups 1, 6, and 7 were also used. Although antibodies were produced to several *L pneumophila* serogroups or *Legionella* species by individuals, there was no constant pattern, suggesting that the response is a characteristic of the infected individual and not of the infecting strain of *Legionella*. There is evidence that heat-killed antigen made from *L pneumophila* serogroup 7 may give unreliable results.

In 1984 an airborne outbreak of infection with serogroup 1 *Legionella pneumophila* originating from a common source occurred in the Dennistoun area of Glasgow.¹ Thirty three cases of certain or probable infection were identified, 26 of whom lived in an area adjacent to the common source. The other seven patients worked in or travelled through the district. Many cases showed a four-fold rise in antibody titre to *L pneumophila* serogroup 1. Others had high (≥ 256) or, in some cases, lower standing titres, together with an illness suggestive of *Legionella* infection. *L pneumophila* serogroup 1 was isolated from two patients, and these strains of Pontiac type 1a² (Tobin JO, personal communication) were indistinguishable from a strain isolated from an evaporative condenser, upwind of the area where cases occurred. Sera were collected from all patients and were examined for the antibody response to a wide range of *Legionella* sp to try and establish whether the antibody response to heterologous legionella antigens observed in cases^{3,4} was a response of the individual or was mediated by the antigenic nature of the infecting strain.

Material and methods

Sera were examined from 32 of the 33 patients, and from eight of these a second serum sample, taken late in the illness, was examined in parallel with the earlier

serum. Most sera were withdrawn one to two weeks after the onset of illness, but from seven patients were taken three to four weeks, and from one, six weeks after onset. Late sera were taken six to 12 weeks after onset. Sera were also taken from 72 people with no history of recent respiratory illness who resided or worked in the Dennistoun area of Glasgow at the time of the outbreak. If not examined immediately, sera were stored at -20°C until examination.

Heat-killed antigens for the indirect fluorescence antibody test (IFAT) were prepared as described previously.⁵ Table 1 shows strains of *Legionella* sp from which antigens were prepared. Strains G13 (from a patient in the outbreak) and G14 (from the evaporative condenser associated with the outbreak) were of Pontiac type 1a, as determined by testing with monoclonal antibodies. Formolised yolk sac antigens (FYSA) made from *L pneumophila* serogroup 1 (Pontiac strain) and serogroup 6 were supplied by the Division of Microbiological Reagents and Quality Control (DMRQC), Central Public Health Laboratory, Colindale. FYSA from serogroup 7 *L pneumophila* was made by the same method as that used at DMRQC.⁶ All antigens were shown to react in immunofluorescence tests with specific antisera produced in rabbits with the exception of serogroup 1 *L pneumophila* antigens, which were controlled using known positive human sera.

The IFAT using heat-killed antigen or FYSA was carried out as previously described,⁵ except that tests using FYSA were read using a water immersion objective, at a magnification of 50 and eyepieces at a magnification of 10.

Table 1 *Legionella* species from which antigens were prepared

<i>L. pneumophila</i>		Other <i>Legionella</i> sp
Serogroup	Strain	
1	Philadelphia 1	<i>L. anisa</i> (CH-47-C-1)
1	G13 (isolated from one of the patients in the outbreak)	<i>L. bozemanii</i> serogroup 1 (WIGA)
1	G14 (isolated from a cooling tower)	<i>L. dumoffii</i> (TEX-KL)
2	Togus	<i>L. feeleii</i> serogroup 1 (WO-44-C-3)
3	Bloomington-2	<i>L. gormanii</i> (L3-13)
6	Chicago-2	<i>L. jordanis</i> (BL-540)
7	Chicago-8	<i>L. longbeachae</i> serogroup 1 (Longbeach-4)
8	Concorde-3	<i>L. longbeachae</i> serogroup 2 (Tucker-1)
9	IN23	<i>L. micdadei</i> (Tatlock)
10	Leiden-1	<i>L. oakridgensis</i> (OR-10)
P185	(an atypical strain of <i>L. pneumophila</i> serogroup 4)	<i>L. wadsworthii</i> (81-716A)
P183	(An unclassified strain)	"Species 1" strains: Los Angeles-1 (formerly designated as <i>L. pneumophila</i> serogroup 4) Dallas 1-E (formerly designated as <i>L. pneumophila</i> serogroup 5)

Figs 1-4 Comparison of antibody titres obtained with 32 sera in indirect fluorescence test using three *L. pneumophila* serogroup 1 heat-killed antigens: Philadelphia 1, G13, G14 and FYSA (Pontiac strain).

Table 2 Antibody response in patients infected with *L pneumophila* serogroup 1

Antigens	Case No.																				Repeat sera from Case No.				
	1	2	3	4	6	7	8	9	10	13	14	15	17	18	20	24	26	28	30	10	12	19	30	31	
<i>L pneumophila</i>																									
2													+				+								
3				+																					
4*				+		+											+		+						
5*																		+							
6		⊕	+	⊕							⊕	○	+	⊕											
7		+	+						+																
8				+		+						+	+				+								
9				+			+					+													
10	+												+												
P183		+	+	+											+										
P185	+	+	+	+	+	+				+			+				+		+	+	+	+		+	
<i>L anisa</i>	+																								
<i>L dumoffii</i>																									
<i>L feeleii</i> 1												+				+									
<i>L gormanii</i>		+	+																						
<i>L jordanis</i>	+	+											+												
<i>L longbeachae</i> 1												+	+	+											
<i>L longbeachae</i> 2	+							+						+							+				
<i>L micdadei</i>	+	+	+												+										
<i>L oakridgensis</i>		+							+				+		+			+				+			
<i>L pneumophila</i> SG1 (Philadelphia 1)								+																	
(actual titre shown)	512	8192	512	8192	1024	512	1024	128	128	512	512	512	8192	2048	512	>512	2048	512	512	2048	256	512	512	8192	

+ = antibody at a titre of ≥ 64 ; 0 = reacted with serogroup 6 FYSA; * = species 1 (table 1).

Results

All patients showed an antibody response to serogroup 1 *L pneumophila* although in six of the 32 cases the titre was no higher than 64. In one of these latter cases urinary antigen was shown by Dr R B Kohler and in another serogroup 1 *L pneumophila* was isolated from sputum.

Fig 1 shows the results obtained using heat-killed *L pneumophila* serogroup 1 (Philadelphia 1 strain) antigen compared with those from FYSA made from *L pneumophila* serogroup 1 (Pontiac strain). Figs 2 and 3 show the results of antibody titrations using G13 and G14 antigens compared with those from Philadelphia 1 antigen. Fig 4 compares G13 and G14. Table 2 shows the numbers of patients showing antibody titres ≥ 64 with antigens from other *L pneumophila* serogroups and other *Legionella* species examined. (Many additional heterologous reactions were seen at titres of 16 or 32 but with heated antigen these would not, of themselves, be regarded as significant). No patients had antibody to *L bozemanii* serogroup 1 or *L wadsworthii*. It was found initially that many patients showed an antibody response to heat-killed antigens made from *L pneumophila* of serogroups 6 and 7. Fresh heat-killed antigens were made, and many of the positive results were not reproduced using these new batches of antigens. The results of comparison of this latter serogroup 6 antigen and serogroup 6 FYSA showed agreement in 28

of the 32 sera. In one instance (case 15) the FYSA showed a high titre but the heated antigen showed a titre of only 16. In the two other sera examined (cases 3 and 17) a raised titre was seen using the heated antigen but not with FYSA. The results with new serogroup 7 antigen using organisms treated at 100°C for an hour showed agreement with FYSA with 28 of the sera in that no antibody was then shown. The remaining three sera showed a raised titre using this heated antigen but not with FYSA. One of these sera (case 3) was one of those which gave a discrepant result also with serogroup 6 FYSA noted above. None of the 72 control sera reacted at a titre of > 32 with any of the antigens tested.

Discussion

It is clear from the results in table 2 that the antibody of patients to strains of *Legionella* other than *L pneumophila* serogroup 1, which was responsible for the outbreak, was a feature of the individual and not of the infecting strain. Hence, no two patients showed the same pattern of antibody response. Heterologous response was commonest to *L pneumophila* strain P185, *L micdadei*, and *L pneumophila* serogroup 6. Some patients showed a broader response than others and most, but not all, of these had high titres of antibody to *L pneumophila* serogroup 1. Three patients with an antibody titre ≥ 512 , however, did not have a broad antibody response. Overall, 19 of the

32 patients showed a heterologous response (at a titre of ≥ 64) in the first specimen and three in the second alone.

This heterologous response was first described with respect to a response to more than one serogroup of *L. pneumophila*.³ The response to antigens of *Legionella* sp other than *L. pneumophila* is not surprising and has often been seen in this laboratory. It can be confusing, especially where the antibody response to *L. pneumophila* is lower than that to another legionella. The poor serological response of one patient (case 25) who had an antibody titre that never rose above 64 to *L. pneumophila* serogroup 1 (despite a classical legionella pneumonia with *L. pneumophila* serogroup 1 antigenuria), is noteworthy. It illustrates that low antibody titres, with a history of a respiratory illness, cannot be dismissed without careful consideration.

The difference in the findings using *L. pneumophila* serogroup 1 FYSA as opposed to heated antigen are of interest. For many years we have found a close correlation between serum antibodies as detected by either antigen with only occasional discrepancies. This outbreak presented an ideal opportunity for a more formal comparison, and the results in fig 1 confirm this impression, there being agreement ($\pm$ one two-fold dilution) with sera from 23 patients and a positive titre in eight more, although in these the antibody titre shown using heat-killed antigen was higher than that with FYSA. In only one instance (case 25) did FYSA fail to recognise antibody (at a serum dilution of 16), which was recognised at a dilution of 64 by heat-killed antigen.

The results using antigens G13 and G14 made from strains isolated in the outbreak are also of interest. On several occasions we have failed to show any advantage using a patient's strain of *L. pneumophila* serogroup 1 as heated antigen over our standard antigen made from the Philadelphia 1 strain of *L. pneumophila* serogroup 1. The results presented here confirm our findings. It is interesting to note that one of the two antigens (G14) failed to react with serum from case 25, even at a dilution of 16. When the results of antibody estimations using G13 and G14 as antigens were compared G14 was less sensitive than G13.

The results obtained with the first antigens made from *L. pneumophila* serogroups 6 and 7 were disturbing. They may well not have been recognised as suspicious if we had not tested a large number of sera with known antibody to *L. pneumophila* serogroup 1 at one time, as sera from persons living in the infected area but without a history of respiratory infection did not react with these antigens. Presumably the strain of *L. pneumophila* responsible for the outbreak stimulated the production of cross reacting antibodies, which, however, gave many fewer positive reactions with FYSA or with antigens using legionella suspensions heated at 100°C for one hour. The reasons

for the initial results are not known and point to the difficulties that may arise in examining patients' sera for antibodies to *L. pneumophila* serogroup other than 1. Our experience with antigens made from other *Legionella* sp emphasises the difficulties of interpretation that arise from time to time, as reported previously.⁷

Examination of second sera from eight patients showed that the four with cross reacting antibodies had lost these but that five (including two with previous cross reacting antibodies) had developed antibodies, mostly to the atypical serogroup 4 strain, P185. The eight patients still had raised titres of antibody to *L. pneumophila* serogroup 1. The response to heterologous antigens would seem to have been due to antigen handling by the individual rather than pre-existing specific antibody—particularly as infection with legionella other than *L. pneumophila* serogroup 1 is rare in Scotland.⁴ The possibility that the antibody response may reflect previous experience with antigens related to those found in the various legionellas examined remains an open question.

Since this work was completed P183 has been identified as a strain of *L. pneumophila* serogroup 9 (Tobin Jo'H, personal communication).

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