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Legionella pneumophila serogroup 1 subgrouping by monoclonal antibodies – an epidemiological tool

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SUMMARY

A panel of 10 monoclonal antibodies was used to subgroup 326 strains of *Legionella pneumophila* serogroup 1. All but two strains could be classified into three major subgroups named after their representative strains Pontiac 1, Olda and Bellingham 1. Of the 50 isolates from patients, 44 representing 32 separate incidents were of the Pontiac subgroup. This subgroup was also found in 16 of 18 buildings epidemiologically associated with Legionnaires' Disease. In contrast, strains of the Olda subgroup predominated in buildings where no infections had occurred. In 9 of the 11 incidents where isolates were available from at least one patient as well as from the suspected environmental source, the monoclonal antibody reaction patterns of strains from patients were identical to those of one or more of their environmental counterparts.

INTRODUCTION

Monoclonal antibodies (MAs) have not yet been employed extensively for typing or grouping bacterial strains within genera or species. *Neisseria gonorrhoea* strains have been classified this way (Tam *et al.* 1982) and *Legionella pneumophila* serogroups have been further divided into subgroups using MAs (McKinney *et al.* 1983; Joly, Chem & Ramsay, 1983; Watkins & Tobin, 1984), with the findings applied to studies in the field (Watkins & Tobin, 1983; Joly & Winn, 1984).

We report here more extensive studies with MAs to *L. pneumophila* serogroup 1 (SG1), the commonest serogroup to cause human disease (Reingold *et al.* 1984). Isolates from single cases or outbreaks of Legionnaires' Disease, and from their proximate environment as well as from water sources unassociated with human disease were compared by sub-grouping.

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MATERIALS AND METHODS

A total of 206 environmental isolates was obtained from the DHSS survey (unpublished) of the distribution of legionella in water systems and cooling towers and from investigations of similar British and other Continental European sources. Strains from 50 patients were received through the kindness of laboratory staff who had been engaged in their isolation. Seventy strains were also received from the United States and Canada, the majority being from an inter-laboratory study to compare results obtained with different sets of monoclonal antibodies to *L. pneumophila* SG1.

The 10 MAs of the subgrouping panel were chosen on the basis of specificities dividing the serogroup, and were selected after somatic cell fusions using five different strains of *L. pneumophila* SG1 as immunogens (Watkins & Tobin, 1984). These strains (Pontiac 1, Bellingham 1 (Washington), Oxford 4032 and JR5, and Kingston 1) were grown on buffered charcoal yeast extract agar (BCYE) at 37 °C in a moist atmosphere. Whole bacterial suspensions, killed by heating at 65 °C for 45–60 min were used to immunize BALB/C mice. Splenocytes from these mice were then fused with P3-NS1-1-Ag. 4-1 mouse myeloma cells by standard procedures (Kohler & Milstein, 1976). The hybridoma cell lines obtained were cultured in RPMI 1640 medium with 10% foetal calf serum. For these studies culture supernatant fluids were used as MA reagents; some of them were produced for us from our cells by Inveresk Research International, Musselburgh, Scotland.

The indirect fluorescent antibody test used for subgrouping was briefly as follows: 10 μl of aqueous bacterial suspensions (10^8/ml), killed by heating at 65 °C for 45–60 min, were spotted on wells of Teflon coated slides (Hendley Ltd.); after drying the slides were fixed in acetone for 10 min; 10 μl of each of a panel of 10 MAs were added to the wells, and the slides incubated at 37 °C for 1·5–3 h, washed twice in phosphate buffered saline (PBS), dried and stained with a 1:70 dilution of an anti-mouse fluorescein conjugate (Miles Laboratories Ltd) for 30 min at 37 °C. After further washing in PBS the slides were mounted in buffered glycerol (pH 8·4) and examined with a Leitz fluorescent microscope ($\times 10 \times 63$) using incident light illumination.

RESULTS

The results of indirect fluorescent antibody tests (as described above) were used to produce a subgrouping scheme (Table 1) for *L. pneumophils* SG1. In the nomenclature of this Table, well known strains (Thomason & Bibb, 1984) have been chosen as representative of both the major and minor subgroups to which the strains belong where possible.

The panel of 10 monoclonal antibodies identified three major subgroups of *L. pneumophila* SG1 each denoted by a classical strain. Thirty-six per cent of the 326 strains were in the Pontiac subgroup, 44% in the Olda subgroup and 19% in the Bellingham subgroup. Only two strains, one of which was from N. America, and which may not be *L. pneumophila*, did not fit into these major groups. The Pontiac and Olda subgroups could be further subdivided by the first seven MAs of the panel (numbers) and each of these again by the last three (lower case letters) giving minor subgroups 1 a, 1 b, 1 c, 2 a, 2 b, etc (Table 1). The third major subgroup, Bellingham,

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Table 1. *Subgrouping of* Legionella pneumophila *serogroup* 1

Major subgroup	Minor subgroup	Monoclonal Antibodies W29	P4C3	P9C3	P13–15	W36	W32	4032	W39	JR5	KIG6
Pontiac	1*	−	−	+	+	+	−	−			
	2	−	+	+	+	+	−	−			
	3	+	−	+	+	+	−	−			
	4	+	+	+	+	+	−	−			
	−a								−	−	−
	−b								+	−	−
	−c								−	+	−
	−d								−	−	+
	−e								−	+	+
Olda	1	+	+	(+)	−	+	−	+			
	2	+	+	(+)	−	+	−	−			
	3	+	−	−	−	+	−	−			
	−a								(+)	+	+
	−b								−	+	−
	−c								−	(+)	(+)
Bellingham		+	−	−	−	+	+	−	+	−	+

* Representative strains, ( ), variable.
Pontiac: 1, Benidorm 030; 2, Cardiff 5; 3, Knoxville 1; 4, Philadelphia 1. *Olda*: 1, Dallas 1; 2, Ann Arbor 6; 3, Porton 1093.

Table 2. *Subgroups of* 50 *clinical isolates of* L. pneumophila *serogroup* 1

Major subgroups	Minor subgroups 1	2	3	4	Total (%)
Pontiac	33	4	5	2	44 (88)
Olda	0	3	0	NA	3 (6)
Bellingham	3	NA	NA	NA	3 (6)

NA, Not applicable.

was homogeneous. Only Pontiac strains amongst the three major subgroups reacted with MA P13-15 and only Bellingham strains reacted with MA W32. The use of 10 monoclonal antibodies facilitated detailed comparisons between the antigenic characteristics of strains, resolving the minor subgroups within the three major ones.

The subgroups of the 50 strains from patients with Legionnaires' Disease acquired in the United Kingdom and other parts of Europe are given in Table 2. Forty-four of these, from 32 separate incidents, were in the Pontiac subgroup of which 33 were in the minor subgroup 1. Three, two of which were from related cases, belonged to the Olda minor subgroup 2, similar to the Ann Arbor 6 strain, and three to the Bellingham subgroup.

The distribution by subgroups of environmental strains is given in Table 3. The establishments which harboured *L. pneumophila* SG1 are divided into those which were either associated with Legionnaires' Disease or not, and by the source of the isolates, either from piped water (i.e. calorifiers, taps, tanks, showers, etc.) or recirculating water systems (i.e. cooling towers of air-conditioning systems).

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Table 3. *Subgroups of* L. pneumophila *serogroup* 1 *found in piped water systems and cooling towers*

	Water systems		Cooling towers	
Subgroups	Case associated establishments ($n = 17$)	Case unassociated establishments ($n = 15$)	Case associated establishments ($n = 5$)	Case unassociated establishments ($n = 27$)
Pontiac	15	1	4	5
Olda 1	3	7	1	13
2	1	2	2	10
Bellingham	3	5	2	0
Others	0	1	0	1

Table 4. *Subgroups of* L. pneumophila *serogroup* 1 *isolates from both patients and associated environments*

	Patient isolates		Environmental isolates		
Incident	No.	Subgroup	No.	Subgroup	Suspected source
A	1	Bell	4	Bell	Water system
B	1	Bell	5	Bell	Water system
C	2	Pont 2a	2	Pont 2a	Water system
D	1	Pont 2a	3	Pont 2a	Water system
E	3	Pont 1a	1	Pont 1a	Cooling tower
F	2	Pont 1a	1	Pont 1a	Cooling tower
			1	Bell	Cooling tower
			1	Olda 2a	Cooling tower
G	1	Pont 1a	1	Pont 1a	Cooling tower
H	1	Pont 1a	1	Pont 4e	Water system
I	1	Pont 3c	1	Pont 4e	Water system
			1	Pont 1a	Water system
			2	Olda 1a	Water system
J	8	Pont 1a	2	Pont 1a	Cooling tower
K	1	Pont 2b	1	Pont 2b	Water system

Pont, Pontiac; Bell, Bellingham.

Fifteen of the piped water systems from 17 buildings epidemiologically associated with the disease yielded strains of the Pontiac subgroup. In comparison, this subgroup was found in only one of 15 unassociated piped water systems. This subgroup was also detected less often than other subgroups in cooling towers not associated with outbreaks. Strains in the Olda subgroups were the predominant ones found in both water systems (9 of 15), and cooling towers (23 of 27) not associated with cases. In 85% of buildings where *L. pneumophila* SG1 was isolated from several sources, only one subgroup was found.

In 9 of the 11 incidents in which *L. pneumophila* SG1 strains were obtained from both patients and the epidemiologically suspected environmental source, the monoclonal antibody reaction patterns of the former strains were identical to one or more of the latter strains (Table 4).

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DISCUSSION

In the United Kingdom, as in North America, the majority of strains causing Legionnaires' Disease belong to *L. pneumophila* SG1 (Reingold *et al.* 1984) This serogroup has been found in a high proportion of water systems and cooling towers in hospitals, hotels and other large buildings. It is therefore important to decide if, as indicated by the Ohio experience, some strains are more likely to cause human disease than others, (Plouffe *et al.* 1984).

Our results with MAs suggest that within *L. pneumophila* SG1, strains of the Pontiac minor subgroup 1 are more likely to cause disease than others (Table 2). It has been reported (Joly & Winn, 1984) that in Vermont, Knoxville-like strains (Pontiac minor subgroup 3 – their group A) caused a large nosocomial outbreak whilst Olda strains (their group B) were responsible for sporadic cases.

In establishments associated with Legionnaires' Disease, strains of the Pontiac subgroup predominated. In environmental sources not associated with human disease these strains were infrequently found (Table 3). In the latter situation, Olda-like strains were the most frequently isolated. No strain of the Olda minor subgroup 1, which was the commonest environmental type found has yet been isolated from patients in Britain. The three human isolates of the Olda subgroup were in minor subgroup 2a or 2b.

Strains of the Bellingham subgroup were found in 18 % of disease-associated piped water systems and 33 % in disease unassociated systems but were isolated from only 3 of the 50 human infections (6 %). Three of 13 establishments (23 %) associated with cases of Legionnaires' Disease where no clinical isolates had been obtained yielded strains of this subgroup, and this suggests that it may cause more cases of illness than the 6 % found in clinical cases indicated.

The reproducibility of subgrouping using our panel of 10 MAs (9 of which recognise antigenic determinants on the lipopolysaccharide of the outer cell membrane) was good, with only an occasional strain failing to react with a MA on repeat testing. Moreover, six representative strains which were repeatedly subcultured on BCYE agar for 4–6 weeks maintained the same pattern of MA reactivity. It is important to note however, that variations in the preparation of the *L. pneumophila* SG1 strains for subgrouping can significantly modify the results. Identical results were obtained using untreated legionellae, or organisms treated overnight with 1 % v.v. formalin or heated at 65 °C for 1 h. Detergent treatment (Gosting *et al.* 1984) can alter results and heating to 100 °C for 15 min causes some reactions to be amplified and others, including that with MA P13–15, to be abolished (I.D.W. unpublished results).

Somatic typing sera prepared by cross-absorption techniques in the classical manner have been used to classify SG1 strains (Thomason & Bibb, 1984). The main advantage MAs have over sera prepared in this way is that less laboratory time is needed to replenish stocks when the originals are exhausted.

These studies show that MAs can be used to subgroup strains within *L. pneumophila* SG1 and also to identify strains more likely to cause disease in man. The similarity of associated human and environmental isolates may be ascertained, thus helping to identify or exclude a source of infection. The two incidents where the clinical and environmental isolates were not identical (Table 4–H & I) were

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from the first two *L. pneumophila* SG1 outbreaks studied in the United Kingdom from which several environmental strains were lost during storage.

The results of a U.S.A./Canada/United Kingdom cooperative study are currently being analysed so that a common nomenclature for *L. pneumophila* SG1 subgroups can be proposed.

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