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Intracellular growth of *Legionella pneumophila* serogroup 1 monoclonal antibody type 2 positive and negative bacteria

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SUMMARY

Epidemiological evidence suggests that monoclonal antibody type 2 positive (MAB 2^+) *Legionella pneumophila* serogroup 1 (LP1) more often causes disease than do MAB 2^- isolates, and there is evidence that MAB 2^- LP1 grow less well in cells than do MAB 2^+ bacteria. We tested the intracellular growth rates of ten randomly selected MAB 2^- LP1 isolates, by using guinea-pig alveolar macrophages, and human monocyte-derived macrophages. Save a low virulence control, all ten MAB 2^- isolates grew as well in cells as a virulent MAB 2^+ isolate. Heterogeneity of MAB 2^- LP1 growth in cells exists, making poor intracellular growth an unlikely explanation for why MAB 2^+ LP1 appear to cause disease more often.

INTRODUCTION

Legionella pneumophila serogroup 1 (LP1) isolates can be divided into those isolates that are either reactive or nonreactive with monoclonal antibody (MAB) 2; those that are MAB 2^+ are often referred to as belonging to the 'Pontiac' subgroup [1]. Several retrospective studies have determined that MAB 2^+ LP1 isolates are the most frequent clinical LP1 isolates, in contrast to their lower frequency in the environment [2–5]. Based on this information, it has been thought that MAB 2^+ LP1 bacteria are more virulent than are MAB 2^- LP1 bacteria. Laboratory studies of limited numbers of MAB 2^- LP1 isolates have demonstrated decreased virulence for animals, decreased growth in macrophages, or decreased aerosol survival [6–9]. It has been hypothesized that MAB 2^- may be less virulent because they multiply less well in macrophages. In this study we screened MAB 2^- LP1 isolates for growth in cultured cells, and we show that reduced growth in macrophages is not a uniform characteristic of these isolates.

MATERIALS AND METHODS

Eleven MAB 2^- LP1 isolates were tested in macrophages, and compared to the growth in macrophages of a MAB 2^+ LP1 strain, F889 (Table 1). Except for a MAB 2^- LP1 strain of known relative avirulence, F2189, all other ten MAB 2^- LP1 isolates were picked at random from our collection of over 1000 environmental

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Table 1. *Growth of* L. Pneumophila *serogroup 1 MAB 2 strains in macrophages* (*log_{10} c.f.u./ml change after 2 days of infection*)

Bacterial		Sources of macrophages		
		Guinea-pig alveolar		Human blood
Strain	Source	Expt 1	Expt 2	
889* (Positive control)	CA1†	1·6 (1·5–1·7)‡	2·4 (2·3–2·4)	1·6 (1·5–1·7)
2189 (Negative control)	OH1	0·9 (0·4–1·1)	1·6 (1·4–1·7)	1·3 (1·0–1·4)
1639	CA2	ND§	2·8 (2·7–2·8)	ND
1789	CA2	2·6 (2·6–2·7)	ND	1·7 (1·5–1·8)
2176	PA1	1·9 (1·8–2·0)	3·1 (3·1–3·2)	ND
2177	PA1	2·2 (2·1–2·3)	ND	ND
2178	PA1	2·3 (2·3–2·4)	ND	ND
2179	PA1	2·0 (1·9–2·1)	ND	1·7 (1·6–1·8)
2180	PA1	2·4 (2·4–2·5)	ND	ND
2181	PA2	2·0 (1·9–2·1)	ND	ND
2183	DL1	2·1 (2·0–2·2)	ND	ND
2184	DL1	1·6 (1·4–1·7)	ND	2·1 (2·0–2·2)

* Strain 889 is MAB 2^+, is a clinical isolate, and of high virulence for guinea-pigs. All other strains are MAB 2^- and environmental isolates; strain 2189 is MAB 2^- and of known low virulence for guinea-pigs.

† Geographical source of strain; CA, California, PA, Pennsylvania, DL, Delaware, OH, Ohio; 1 & 2 are site numbers in each state.

‡ Mean 2 day change, with 95% confidence intervals shown in parentheses.

§ ND, not done.

LP1 isolates. Bacterial strain F889 is a clinical isolate that is virulent for guinea-pigs [10]. Bacterial strain F2189, also known as RH1, was obtained from Michael Para, Ohio State University; this strain is relatively avirulent for guinea-pigs [6]. Bacteria were grown on buffered charcoal yeast extract agar, supplemented with 0·1% α-ketoglutarate (BCYEα), for 1–2 days at 35 °C in air [11]. For infection of cell cultures, bacteria were harvested from BCYEα plates, using normal saline, then washed two to three times in the same solution. The bacteria were diluted in RPMI 1640, or Medium 199 with Earles' balanced salt solution (M 199) (JRH Biosciences, Lanexa, Kansas), to approximately 1×10^5 c.f.u./ml just prior to testing. Actual numbers of bacteria added to cell cultures were determined by viable plate counting in duplicate, using BCYEα agar.

Monoclonal antibody typing of LP1 was performed by using indirect immunofluorescence with MAB 415 (MAB 2-reactive) (M. McIntyre, Oxford Public Health Service), or with MAB 2 (ATCC CRL 1770). Some of the isolates had been serotyped previously by John Tobin [12]. In all cases the previously determined serotype identity of the strains was reconfirmed. The serotyping protocol was performed as described previously, using MAB 2^- and MAB 2^+ LP1 strains as controls (ATCC 43106, 43107, 43108, 43109, 43110, 43111, 43112, 43113) [1].

Guinea-pigs' alveolar macrophages were harvested by using bronchoalveolar lavage as described previously [10]. The macrophages were purified by plating in Linbro 24-well tissue culture plates (Flow Laboratories Inc., McLean, Virginia), as described previously [10], and eventually resuspended in M 199 supplemented

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with 20% fetal calf serum ($2{\cdot}5 \times 10^5$ macrophages per well). Plated macrophages were infected on the day of their harvest.

Human mononuclear cells were separated from other blood components by density-gradient centrifugation of heparinized blood diluted in Histopaque 1077 Hybri-Max (Sigma Chemical, St Louis, Missouri). The blood donor had an antibody titre to *L. pneumophila* serogroup 1 of $\leqslant 64$ [13]. Monocytes were further purified by plating on gelatin, which was extensively washed to remove lymphocytes, and then washed with 5 mM-EDTA to remove the monocytes (90% purity and viability) [14]. The monocytes were washed twice thereafter, resuspended in M 199 with 30% fresh autologous serum, and then incubated for 40 h in Linbro 24-well tissue culture plates (final concentration $\approx 2 \times 10^5$ monocytes/well). The cells were washed thrice with M 199, after which was added M 199, with 10% fresh autologous serum.

Legionella pneumophila bacteria were added to guinea-pig alveolar macrophages or human monocyte-derived macrophages with a bacteria-to-cell ratio of $\approx 1:10$. After infection, the tissue culture plates were shaken for 60 min at 50 rev./min at 37 °C in 5% CO_2; subsequently they were incubated for 2 days in stationary culture under the same conditions. Bacteria were quantified by plating serial dilutions of tissue-culture supernate in duplicate on BCYEα agar at the initiation of infection and 1–3 days thereafter. No extracellular growth of *L. pneumophila* occurs under these conditions, so *L. pneumophila* concentrations within the supernate represent both the original extracellular inoculum and multiplying intracellular bacteria that have lysed the macrophages [15]. Changes in supernate concentrations of bacteria are due only to intracellular growth.

RESULTS

The growth of the LP1 isolates tested in macrophages is shown in Table 1. Only the known low-virulence strain, F2189, grew consistently less well than did F889 or the other MAB 2^- isolates tested.

DISCUSSION

Contrary to expectation, our MAB 2^- isolates grew as well in two different types of cells as did the MAB 2^+ isolate that we tested. Sampling error is a possible explanation of our inability to find MAB 2^- bacteria that grow poorly in cells, but is unlikely, since four different geographical regions were the sources of the study isolates. Loss or change of the MAB 2 epitope, resulting in testing strains that were previously MAB 2^+ but are now MAB 2^-, is unlikely, as we and others have observed the stability of this marker with serial passage [1, 12]. However, there is one report of the variable expression of this epitope [16]. Our cell-infection assays were able to detect the known reduced virulence of bacterial strain F2189, confirming our ability to detect at least one type of reduced cell infectivity. Our findings are contrary to those of Dournon and colleagues, who found that all three MAB 2^- LP1 isolates that they tested infected human monocytes less well than did MAB 2^+ LP1 bacteria, and that four MAB 2^- strains were less virulent for guinea-pigs than were MAB 2^+ strains [8, 9]. Aside from possible strain and

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methodological differences, we have no explanation for the discrepant results. Our findings suggest two possibilities: (*a*) environmental isolation of MAB 2^+ LP1 is more difficult than its clinical isolation; (*b*) tests of the relative ability of LP1 strains to infect macrophages are not sensitive to major virulence differences for humans. At the very least, MAB 2^- LP1 strains are heterogeneous in their ability to infect cells.

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