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Protection against *Brucella abortus* in Mice with O-Polysaccharide-Specific Monoclonal Antibodies

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Mice injected with either of two monoclonal antibodies specific for the O polysaccharide of *Brucella abortus* prior to challenge infection had viable counts in spleens and livers significantly below those in control groups 1 and 4 weeks later. Two monoclonal antibodies specific for the porin of *B. abortus* failed to confer protection.

The current understanding of *Brucella abortus* as an intracellular pathogen and of protective immunity to brucellosis is based largely on studies of mice (4, 7, 14, 18). There is good evidence that passively transferred antibodies confer a measure of immunity to *B. abortus* infection in mice (3, 15, 17, 19, 23), yet the specificity of protective antibodies has not been rigorously established. In this study, we tested four monoclonal antibodies (MAbs) specific for the *B. abortus* O polysaccharide or porin for passive protection of mice to *B. abortus* infection.

Hybridomas producing MAbs against *B. abortus* antigens were prepared from splenocytes of BALB/c mice immunized with irradiated cells of *B. abortus* smooth strain 2308 (24) as described previously (11). Supernatants from microtiter wells containing hybridoma growth were analyzed for anti-brucella antibody activity by a solid-phase enzyme-linked immunosorbent assay (ELISA) with alkali-treated lipopolysaccharide (LPS) (16) or porin (24) of *B. abortus* 2308 as the antigen. Selected hydridomas were recloned three times by limiting dilution and grown to extinction in serum-free medium (HB101; Hana Biologics, San Diego, Calif.). Serum-free medium supernatants were concentrated to 10.0 mg of immunoglobulin per ml and isotyped with anti-mouse isotype-specific ELISA procedures (Table 1). In nitrocellulose electroblots of sodium dodecyl sulfate-polyacrylamide gels of a whole-cell extract of strain 2308 MAbs 144 and 145 reacted with a single band corresponding to the porin (24), whereas MAbs 34 and 47 reacted with the typical multiple bands of LPS (22), and MAb 135 failed to react with any antigen (Table 1). Inhibition tests were performed with purified O antigen (25), which had been established to be a perosamine homopolymer by nuclear magnetic resonance analysis in confirmation of a previous report (6), and purified porin electroeluted from sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, which migrated as a single band in sodium dodecyl sulfate-polyacrylamide gel electrophoresis in accordance with published reports (24). Competitive inhibition assays of the nitrocellulose blots demonstrated that 25 μg of purified porin inhibited a 1:200 dilution of MAbs 144 and 145, whereas 10 μg of purified O antigen inhibited a 1:200 dilution of MAbs 34 and 47. In addition, competitive inhibition ELISAs (25) were performed with soluble strain 2308 antigen (12) attached to the solid phase. The purified O antigen inhibited MAb 34 86%, MAb 47 88%, MAb 144 4%, and MAb 145 6%. Purified porin inhibited MAb 34 9%, MAb 45 6%, MAb 144 89%, and MAb 145 81%.

Female 10-week-old Balb/cByJ mice (Jackson Laboratory, Bar Harbor, Maine) were used throughout the study. After an 18-h fast, groups of five mice were injected intraperitoneally (i.p.) with 100 μg of MAb (in 0.1 to 0.5 ml of serum-free tissue culture supernatant) or 0.5 ml of sterile phosphate-buffered saline (PBS). Four hours later, mice were inoculated i.p. with 5×10^4 CFU of virulent *B. abortus* 2308 in 0.1 ml of PBS. To prepare the challenge suspension, a 48-h culture on Schaedler blood agar (24) was suspended in sterile PBS and adjusted turbidimetrically to 5×10^5 CFU/ml. The exact dose was calculated retrospectively by viable counts. At 1 or 4 weeks postinfection (p.i.), mice were killed by cervical dislocation, and spleens and livers were removed aseptically. The organs were homogenized in 10 ml of PBS with a Sorvall Omni-Mixer (DuPont Co., Wilmington, Del.). Serial 10-fold dilutions were plated on Schaedler blood agar, and colonies were counted after 3 days of incubation at 37°C in air containing 5% CO_2.

In a preliminary experiment, the effect of MAbs were tested on the course of infection after 1 week in the spleen (Table 2). Statistical significance was assessed with a Bonferroni multiple-comparison *t*-test procedure at an experimentwise error rate of 5% (21). Analysis of variance revealed no significant differences in counts between the groups receiving PBS and control antibody 135 or those receiving O antibodies 34 and 47, so the data from those pairs were pooled for further analyses. O antibodies reduced viable counts by more than 2 logs ($P < 0.0005$). Counts obtained from groups treated with porin antibodies differed from one another ($P < 0.05$), although neither differed significantly from the pooled control. Plommet and Plommet (19) tested the protective effects in mice of polyvalent antisera directed against the crude LPS or sodium dodecyl sulfate-insoluble extract (PG). Both types of antisera, administered intravenously simultaneously with the challenge inoculum, caused a shift of infection from spleen to liver detectable up to 7 days p.i., so that bacterial numbers in livers of passively immunized mice in fact exceeded those in control groups (19). By p.i. day 21, PG antisera had produced a progressive decrease in spleen counts, whereas spleen counts in mice treated with LPS antisera differed little from those at 7 days (19). To examine these questions in our system, were injected mice i.p. with 100 μg of O-specific MAbs and enumerated *B. abortus* in spleens and livers at 1

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TABLE 1. Properties of MAbs

Clone	Specificity for *B. abortus*	Isotype	Titer[a]			K_a[e]
			Agglutination[b]	Complement fixation[c]	ELISA[d]	
135	None[f]	IgG1(κ)	<5	<5	<5	
144	Porin	IgM(κ)	<5	<5	800	6.0×10^9
145	Porin	IgM(κ)	<5	<5	1600	6.2×10^9
34	O polysaccharide	IgG2a(κ)	100	256	>1,600,000	7.6×10^{10}
47	O polysaccharide	IgG3(λ)	<5	16	25,600	8.9×10^9

[a] Based on an initial concentration of 10.0 mg/ml.
[b] Standard plate agglutination test (2).
[c] Standard tube cold complement fixation test with a whole-cell antigen (1).
[d] Microtiter assay (5) using porin with MAbs 144 and 145, O polysaccharide with MAbs 34 and 47, and both antigens with MAb 135.
[e] Relative estimated association constant expressed as liters per mole per molecule of IgM or IgG based on Scatchard plot analysis.
[f] Produced no reaction against any *B. abortus* antigen tested.

TABLE 2. Effect of passive immunization of mice with MAbs on spleen colonization by *B. abortus* 1 week after infection

Antibody[a]	Log_{10} brucella in spleen ± SD
None[b]	6.44 ± 0.14
135	6.44 ± 0.20
144	6.78 ± 0.33
145	5.94 ± 0.37
34	4.00 ± 0.41
47	3.75 ± 0.72

[a] One hundred μg injected i.p. 4 h before i.p. infection with 5×10^4 CFU of *B. abortus*.
[b] Injected with 0.5 ml of PBS.

TABLE 3. Effect of passive immunization of mice with MAbs on spleen and liver colonization 1 and 4 weeks after infection

Antibody[a]	Log_{10} brucella ± SD			
	Spleen		Liver	
	1 week	4 weeks	1 week	4 weeks
None[b]	6.41 ± 0.25	6.47 ± 0.28	4.90 ± 0.24	4.79 ± 0.47
135	6.56 ± 0.41	6.42 ± 0.53	4.71 ± 0.48	5.12 ± 0.39
34	3.34 ± 0.54	4.65 ± 1.18	3.29 ± 0.67	2.78 ± 0.93
47	2.50 ± 0.53	5.69 ± 0.61	2.76 ± 0.38	3.63 ± 1.03

[a] One hundred μg injected i.p. 4 h before i.p. infection with 5×10^4 CFU of *B. abortus*.
[b] Injected with 0.5 ml of PBS.

and 4 weeks p.i. (Table 3). Again, no significant differences in counts occurred at either period in livers or spleens between the two groups injected with O-specific MAbs and the two control groups, so data were pooled. Immunization with O-specific MAbs significantly reduced the numbers of brucella in spleen ($P < 0.0005$ at 1 week and $P < 0.005$ at 4 weeks p.i.) and liver ($P < 0.0005$ at 1 and 4 weeks p.i.). The differences in spleen counts between immunized and control groups diminished between 1 and 4 weeks ($P < 0.0005$), whereas corresponding differences in liver counts were not significantly altered. This is in general accord with Plommet and Plommet, who observed an early but not a late protective effect due to LPS antibodies (19). Our failure to observe early deflection of infection into the liver (19) might be explained in part by differences in route and timing in the administration of antibody and challenge inoculum. Moreover, in our experiment, counts in livers of control groups differed little between the two periods and were 1 to 2 logs below those in the spleen (Table 3). In contrast, Plommet and Plommet (19) reported counts in livers of control animals at 1 week p.i. that were much higher than those in spleens, with a marked reduction between 1 and 3 weeks. The reasons for these differences are unclear, although a principal contributing factor may have been the difference in route of infection.

In recent reports, protection has been conferred on mice by MAbs specific for the LPS of *Pseudomonas aeruginosa* (20), *Escherichia coli* O111:B4 (13), and *Salmonella typhimurium* (8), although in the latter study little success was achieved with the inherently susceptible C3H/HeJ strain in accord with Eisenstein et al. (9). The protective efficacy of MAbs varied and could be correlated with activity in ELISA (8) or agglutination (20) tests. The O-specific MAbs used in our study differed substantially in their serological activities and association constants (Table 1), yet their efficacies were generally similar (Tables 2 and 3). Dose titrations may reveal differences in the protective capacities of MAbs 34 and 47 which were not apparent in this study.

The failure of MAbs specific for porin to provide protection may have been a consequence of their inabilty to fix complement on whole cells (Table 1). MAbs of immunoglobulin M (IgM) or IgG isotype which were specific for outer membrane proteins of *P. aeruginosa* provided some protection but were much less effective than those specific for LPS (20). Protection of infant rats against *Haemophilus influenzae* type b with an MAb specific for an outer membrane protein has also been reported (10).

The results reported here provide the basis for a more detailed analysis of the role played by antibodies in immunity to *B. abortus*. Findings to date are consistent with the hypothesis that O antibodies are an important component in protective immunity and serve to complement the cell-mediated immune response.

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