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Capacity of Passively Administered Antibody To Prevent Establishment of *Brucella abortus* Infection in Mice

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In contrast to immunity against some other facultative intracellular parasites, protective immunity against *Brucella abortus* is mediated in mice by antibodies as well as by cell-mediated immune responses. It was the purpose of this study to determine whether antibody alone would prevent infection with *B. abortus*. The majority (82%) of CD-1 outbred mice infected with 100 CFU of virulent *B. abortus* 2308 preincubated with graded quantities of an O polysaccharide-specific IgG2a monoclonal antibody (MAb) were free of infection 1, 2, 4, and 6 weeks later, based on detection limits of 13 brucellae per spleen and 39 per liver. Infection was present in 95% of control animals. Similar results were obtained with a challenge dose of 500 CFU, but with a challenge dose of 5,000 CFU, infection became established even with the highest concentration of MAb used (50 μg of MAb per 5,000 brucellae). Pretreatment with an O polysaccharide-specific IgG1 MAb or with convalescent-phase serum diminished but did not prevent establishment of infection by 100 CFU of *B. abortus*. A majority of culture-negative mice tested 6 weeks after infection were serologically negative, which could have signified either the absence of previous infection or the early elimination of infection. In an in vitro test system, all of the antibody preparations were efficient in opsonizing *B. abortus*. Effective killing of the organism by unelicited mouse peritoneal macrophages occurred in conventional but not in endotoxin-free medium, suggesting that activated macrophages were required for killing of opsonized *B. abortus*. These results emphasize the potential importance of antibodies in the immunoprophylaxis of brucellosis and suggest that the design of a successful vaccine will require the induction of antibodies not only of appropriate specificity but also of the optimal isotype for mediating protective functions.

Although it has been demonstrated repeatedly in the murine model that passively administered antibody enhances protective immunity against the facultative intracellular bacterium *Brucella abortus* (1, 13–16, 19, 22, 26), antibody titers induced by vaccines in cattle continue to be regarded as undesirable because they interfere with the interpretation of serodiagnostic tests. However, if it were possible to demonstrate that antibodies alone could not only diminish but also prevent the establishment of *B. abortus* infection in mice, there would be cause to examine more critically the role of humoral immunity in bovine brucellosis and perhaps to reevaluate some of the criteria placed on the composition of an ideal vaccine.

This study examined further the role of passive antibody in murine brucellosis. It was our intention to determine whether, under appropriate experimental conditions, antibody was capable of preventing infection with *B. abortus*. Monoclonal antibodies specific for the O polysaccharide were selected because such antibodies had been shown to enhance protection (13, 16). In vitro phagocytosis and killing assays were performed with the same antibodies for comparison with the in vivo experiments. Functional comparisons were also made between the monoclonal antibodies and immune serum from convalescent mice.

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MATERIALS AND METHODS

Mice. BALB/c ByJ mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, and outbred CD-1 mice were obtained from Charles River Breeding Laboratories, Inc., Kingston, N.Y. Females were purchased at 9 weeks of age and kept for 1 week before use.

Bacterial strains. Virulent *B. abortus* 2308 was used in all experiments. For mouse challenge infections, frozen stock cultures of predetermined concentration were diluted directly to the desired level in phosphate-buffered saline (PBS) and injected intravenously. For in vitro challenge of murine macrophages, 2-day plate cultures were suspended in PBS and were adjusted turbidimetrically to the desired concentration (15). Exact doses for in vivo and in vitro experiments were established retrospectively by plate counts (15).

Antibodies for passive transfer. Two murine monoclonal antibodies (MAb) with specificity for the *B. abortus* O polysaccharide were used. An immunoglobulin G2a (IgG2a) MAb produced by clone 34 (16) was grown in tissue culture and had been freed of antibiotics by affinity chromatography and filter sterilized. It was held at 4°C at a final concentration of 2.67 mg/ml. Antibodies produced by clone 34 had been demonstrated previously to confer protection upon mice (16). The antibodies were of high affinity (K_a = 7.6 × 10^{10}/mol) and reacted strongly in agglutination and complement fixation tests and the enzyme-linked immunosorbent assay (16).

An IgG1 MAb specific for the A epitope of the *B. abortus* O polysaccharide (6) was received as 1 ml of lyophilized ascitic fluid. The preparation was reconstituted to 10 ml with

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PBS, dialyzed in PBS for 24 h to remove NaN_3, filter sterilized, and frozen in 1-ml aliquots at −70°C.

Polyclonal antiserum consisted of pooled sera from two mice which had been infected 9 weeks previously with 5×10^4 live cells of *B. abortus* vaccine strain 19. The serum was filter sterilized and frozen at −20°C prior to use. Samples used for in vitro assays were heat inactivated at 56°C for 30 min.

Mouse studies. Mice were given 100 μl of a bacterial suspension by injection into the tail vein. In protection experiments the bacterial suspension had been premixed with antibody or PBS on a rotator for 30 min at 37°C. Mice were anesthetized with Metofane (Pitman-Moore, Inc., Washington Crossing, N.J.) before being exsanguinated by cardiac puncture and cervical dislocation. The spleen and, in most experiments, the left lateral lobe of the liver were removed, homogenized, and cultured quantitatively by methods previously described (15). Individual serum samples were frozen at −20°C until tested for antibodies.

Macrophage studies. Methods for assessment of opsonization and killing in vitro followed in essentials the protocol of Spitalny and North (24). Cells were removed from the peritoneal cavities of normal CD-1 mice by washing, pooled, and allowed to settle for 2 h onto circular cover slips (diameter, 13 mm; Clay Adams, Parsippany, N.J.) in petri dishes (35 by 10 mm; Becton Dickinson Labware, Oxnard, Calif.) overlaid with 1 ml of medium. Nonadherent cells were removed after a 2-h incubation at 37°C under 5% CO_2, and adherent cells (approximately 1×10^5 per cover slip) were incubated for another 1 h prior to the addition of bacteria. A 1-ml volume of a fresh suspension of *B. abortus* 2308 which had been opsonized for 30 min at 37°C was added to each plate. In most experiments the final concentration of *B. abortus* was approximately 10^8 per ml, and a period of 15 min was allowed for phagocytosis. Individual cover slips were then washed thoroughly, placed into new plates with fresh medium, and reincubated. At this time (time zero) and at periodic intervals thereafter, triplicate sets of cover slips were washed, subjected to three cycles of freezing in a dry-ice–alcohol bath and thawing in a 37°C water bath (12), and cultured quantitatively. It had been established that this treatment caused complete lysis of eucaryotic cells without affecting the viability of *B. abortus*. Medium was changed in each plate at 2-h intervals throughout the experiment. Cultures were maintained in antibiotic-free RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) containing 5% heat-inactivated (56°C for 1 h) fetal bovine serum (Sigma Chemical Co., St. Louis, Mo.) and 5 U of preservative-free heparin per ml. Washes were performed with sterile saline containing 0.5% heat-inactivated fetal bovine serum. Concentrations of endotoxin in the RPMI 1640 and the fetal bovine serum were 2.0 and <6.0 endotoxin units (EU/ml, respectively, as determined by the *Limulus* lysate assay (LAL 20 Test Kit; Whittaker Bioproducts, Walkersville, Md.). Selected experiments were performed with endotoxin-free RPMI 1640 (<0.01 EU/ml; Whittaker) containing the same fetal bovine serum at a concentration of 5%. In experiments in which endotoxin-free RPMI 1640 was used, the saline solution used for washing was prepared with endotoxin-free deionized distilled water (<0.02 EU/per ml; Whittaker) and glassware was freed of pyrogens by baking at 170°C for 4 h.

Enzyme immunoassay. Antigen-coated microdilution wells (Nunc, high antigen binding; InterLab, Thousand Oaks, Calif.) were prepared by the addition to each well of 100 μl of f6, a purified preparation of smooth lipopolysaccharide (LPS) from *B. abortus* (20, 30), at 2.5 μg/ml in carbonate buffer (pH 9.6). Coated plates were frozen and then thawed 1 to 2 h before use. Washing consisted of four 200-μl rinses with PBS containing 0.05% Tween 20. External dilutions of serum were made in a solution of Tris-buffered saline (0.02 M Tris [pH 8.0]) containing 0.05% Tween 20 and 0.01% NaN_3. A 100-μl quantity of each test serum was added in duplicate, and the mixtures were incubated overnight at 4°C. After washing, 100 μl of alkaline phosphate-conjugated goat anti-mouse $F(ab')_2$ (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) was added for a 3-h incubation at room temperature. After washing, 100 μl of the substrate *p*-nitrophenyl phosphate (Sigma) was added at 1 mg/ml in diethanolamine buffer (pH 9.8); then the mixture was alternately shaken and read at 405 nm three times at 2-min intervals on a plate reader (model 311; Biotek). Slope values were obtained for each test sample by using the linear regression function of Lotus 1-2-3. A linear relationship has been demonstrated between antibody titers and the log of the slope value (30). Three prediluted standards were included on each plate and used to normalize readings for comparisons among plates.

Statistical methods. A mean value for each bacterial count was obtained by averaging the triplicate values following log conversion (15). Liver counts were adjusted to account for culturing only the left lateral lobe, which made up one-third of the organ. Where required, sufficient quantities of tissue homogenates were plated to permit limits of detection estimated at 13 colonies per spleen and 39 colonies per liver. Thus, spleens and livers in which no colonies were detected in any of the triplicates were assigned numbers of 12 and 38, respectively. Statistical significance was determined by a one-way analysis of variance. Individual comparisons were made with paired one-way *t* tests (23).

RESULTS

Passive protection experiments. Preliminary experiments in which *B. abortus* was opsonized with the IgG1 MAb demonstrated that infection could be reduced but not prevented with the challenge dose (5×10^4 organisms) conventionally used in this laboratory (15, 16, 29, 30). Lower challenge doses were therefore evaluated in BALB/c and CD-1 mice to determine the lowest dose which would establish a chronic infection. The level of the plateau phase decreased with lower challenge doses, and *B. abortus* grew to somewhat smaller numbers in spleens of CD-1 mice. Based on these results, most subsequent experiments were performed with an infection dose of 100 CFU in CD-1 mice. It must be emphasized that over the course of a year, a number of experiments that were initiated with this low infection dose showed unacceptably low levels of infection in a majority of control mice of a given shipment. This occurred with both strains of mice, despite procurement of mice from the same vendors, the use of the same stocks of bacteria for infection, and every effort to keep environmental conditions constant. Lot-to-lot variations in mice, resulting in variable responses to vaccination and challenge infections, have been noted previously (30).

An initial trial with an infection dose of 100 CFU opsonized with three concentrations of the IgG2a MAb (50, 10, and 1 μg/100 μl containing 100 CFU) demonstrated excellent protection at 1 and 2 weeks postinoculation (p.i.) (data not shown). This experiment was repeated and extended to a period of 6 weeks. Although 19 of 20 control mice (95%) were infected at the four time points tested (1, 2, 4, and 6

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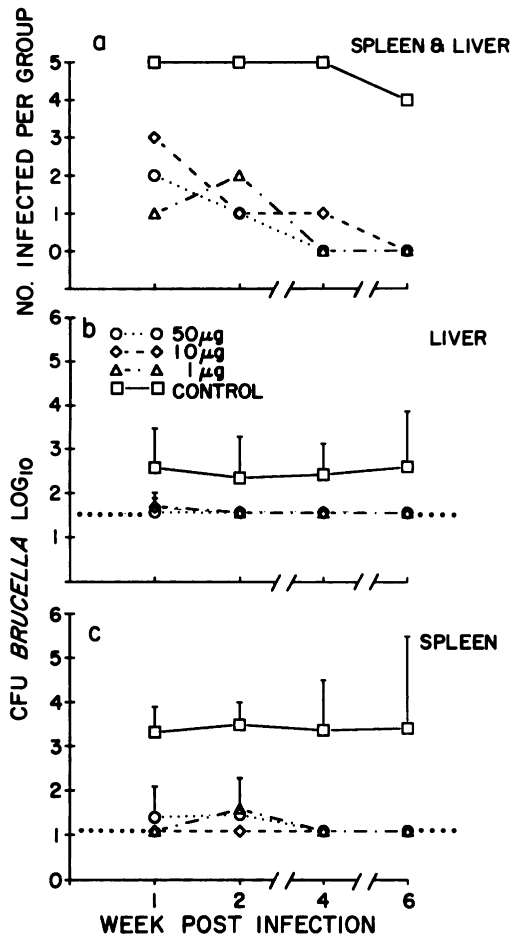

FIG. 1. Growth of *B. abortus* 2308 in livers and spleens of CD-1 mice infected with approximately 100 CFU of unopsonized organisms or organisms opsonized with graded quantities of an IgG2a MAb. There were five mice per treatment group; bars indicate standard deviations. Dotted lines at $\log_{10}$ 1.59 and $\log_{10}$ 1.12 represent the limit of detection of organisms in the liver and spleen, respectively.

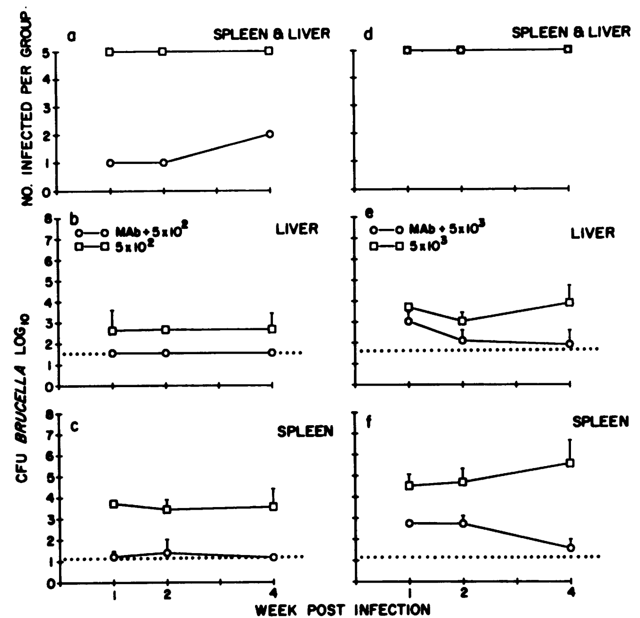

FIG. 2. Growth of *B. abortus* 2308 in livers and spleens of CD-1 mice infected with approximately 500 (a, b, and c) or 5,000 (d, e, and f) CFU in 100 µl, unopsonized or opsonized with 50 µg of an IgG2a MAb. There were five mice per treatment group; bars indicate standard deviations. Dotted lines at $\log_{10}$ 1.59 and $\log_{10}$ 1.12 represent the limit of detection of organisms in the liver and spleen, respectively.

weeks), only 11 of 60 antibody-treated mice (18%) had detectable infections in the liver or spleen (Fig. 1). A mouse was considered infected if a single colony was isolated from either of the organs cultured (Fig. 1a). There was no apparent difference among treatment groups in the total number of mice infected over the course of the experiment. Moreover, the numbers of organisms isolated from the antibody-treated, infected mice were extremely small (Fig. 1b and c), and those isolated from the spleen were significantly smaller than those of the control groups at each time point ($P < 0.05$ to $P < 0.001$). In the liver differences were significant at 1 week p.i. (50-µg group) and 4 weeks p.i. (all groups) ($P < 0.05$).

The effectiveness of the IgG2a MAb against higher infection doses (500 and 5,000 CFU) was evaluated at 1, 2, and 4 weeks p.i. by using the highest concentration of antibody (50 µg/100 µl containing 500 or 5,000 organisms, respectively) used in the previous experiment. At the lower infection dose, only 4 of the 15 antibody-treated mice (27%) were infected (Fig. 2a). Detectable infections were restricted to the spleen (Fig. 2b and c), in which the numbers of organisms cultured were extremely small and differed significantly from those in the control groups (spleen, $P < 0.01$ to $P < 0.001$). At a challenge dose of 5,000 CFU all mice were infected (Fig. 2d), although in antibody-treated mice the numbers of brucellae recovered from the livers and spleens were significantly smaller (spleen, $P < 0.001$; liver $P < 0.05$ to $P < 0.01$) at each time point, and infections were nearly undetectable by 4 weeks p.i. (Fig. 2e and f).

The IgG1 MAb was evaluated in CD-1 mice by using three 10-fold dilutions of antibody (containing 10, 1, and 0.1 µl of reconstituted MAb per 100 µl) and an infecting dose of 100 CFU. There was a significant reduction in spleen counts of antibody-treated mice in all three treatment groups at 1 week p.i. and in two of the three groups at 2 weeks p.i. ($P < 0.01$ to $P < 0.001$) (Table 1). However, the number of infected mice increased in each group from week 1 to week 2 p.i., and 14 of the 15 antibody-treated mice (93%) were infected by week 2 (Table 1).

An experiment of the same design was conducted with convalescent-phase serum from mice which had been infected with strain 19. CD-1 mice were injected with 100 brucellae which had been opsonized with antiserum in dilutions of 1:5, 1:50, or 1:500. At weeks 1 and 2 p.i., 29 of 30 mice (97%) given opsonized bacteria were infected, whereas at 4 and 6 weeks p.i., the number of infected mice in the treated groups had decreased to 5 of 30 (17%) and clearance was complete in the groups which had received cells opsonized with the intermediate dilution of antiserum (Fig. 3a). Bacterial counts in the spleens of antibody-treated mice were significantly lower ($P < 0.05$ to $P < 0.001$) in all groups at all time points, with the exception of the group given the 1:500 dilution at 6 weeks p.i. (Fig. 3c). In accord with prior observations (21), liver counts of antibody-treated mice in two of three groups (1:5 and 1:500) were significantly higher than those of control groups at 1 week p.i. ($P < 0.05$),

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TABLE 1. Effect of opsonization of *B. abortus* with an IgG1 MAb on the establishment of infection in mice[a]

Week p.i.	Amt of MAb per mouse[b] (μl)	Log_{10} brucellae in spleens (mean ± SD)[c]	No. infected per group[d]
1	10.0	1.33 ± 0.51***	1
	1.0	1.54 ± 0.60**	3
	0.1	1.74 ± 0.60**	3
	0	3.13 ± 0.28	5
2	10.0	1.46 ± 0.34***	4
	1.0	1.85 ± 0.54**	5
	0.1	2.99 ± 0.23 (NS)	5
	0	3.40 ± 0.48	5

[a] Infection was performed intravenously with approximately 100 CFU of *B. abortus* 2308 following incubation for 30 min at 37°C with graded concentrations of MAb.
[b] Quantities represent 1:10 dilutions of ascitic fluid in PBS. Thus, 10 μl would contain 1.0 μl of undiluted ascitic fluid.
[c] In comparisons with untreated control groups: ***, $P < 0.001$; **, $P < 0.01$; NS, not significant.
[d] There were five mice per group.

but counts of all treated groups were significantly lower ($P < 0.05$) at 4 weeks p.i. (Fig. 3b).

Antibody responses following infection. Antibody responses and spleen counts of individual mice infected 6 weeks previously with *B. abortus* pretreated with the IgG2a MAb or with polyclonal antiserum are presented in Tables 2 and 3, respectively. Of 15 mice in each group infected with opsonized bacteria, 9 displayed antibody responses comparable to those in normal mice (slopes, ≤16). All of these were culture negative (Tables 2 and 3). The remaining 12 animals had moderate to high antibody responses in conjunction with culture-negative (10 mice) or culture-positive status (Tables 2 and 3). The antibody levels of the five mice treated with the 1:5 serum dilution were quite uniform and may have represented residual levels of transferred antibodies (Table 3). Of 12 control mice, 11 were culture positive and all had moderate to high antibody responses. However, there was no consistent relationship between the numbers of brucellae in the spleen and the level of the antibody response (Tables 2 and 3).

Opsonization and killing assays in vitro. Results of representative experiments are shown in Fig. 4. Ingestion of *B. abortus* 2308 was negligible in the absence of antibodies but was effectively facilitated by both of the MAbs and by the polyclonal antiserum used in the passive-transfer experiments (Fig. 4). The total number of brucellae ingested was related directly to the concentrations of bacteria and antibodies used and to the time permitted for phagocytosis (data not shown). A time of 15 min was selected for phagocytosis to prevent an underestimation of intracellular killing, because in conventional medium up to 30% of the ingested brucellae were killed within the first hour (data not shown). When concentrations of opsonizing antibodies were adjusted to permit an approximately equivalent uptake of *B. abortus*, bacterial killing in conventional medium was efficient and followed similar kinetics with all three preparations. By 4 h after phagocytosis, 90% or more of the ingested organisms had been killed (Fig. 4a). In endotoxin-free medium, on the other hand, phagocytosis was uniformly diminished and the rates of killing were dramatically reduced, in particular following opsonization with whole serum when initial replication occurred (Fig. 4b).

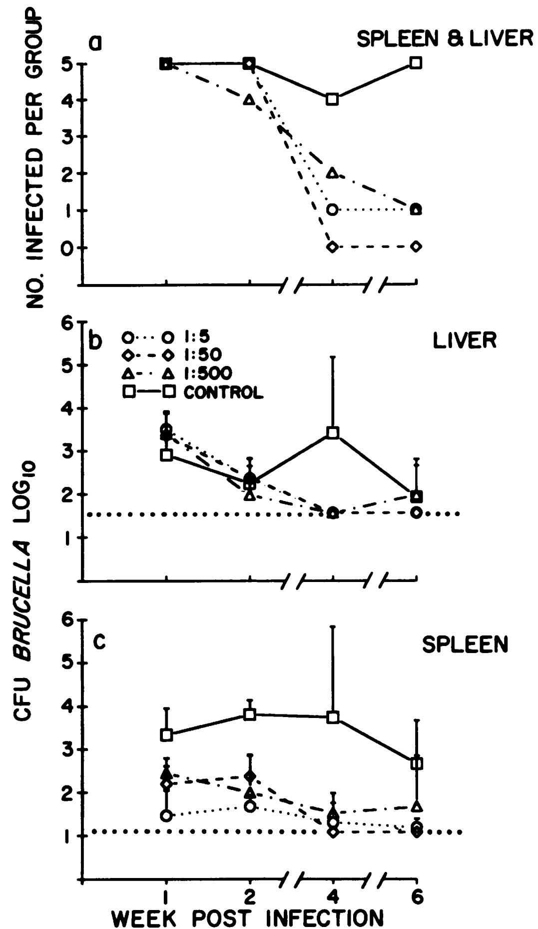

FIG. 3. Growth of *B. abortus* 2308 in livers and spleens of CD-1 mice infected with approximately 100 CFU of unopsonized organisms or organisms opsonized with graded quantities of a polyclonal antiserum. There were five mice per treatment group; bars indicate standard deviations. Dotted lines at $\log_{10}$ 1.59 and $\log_{10}$ 1.12 represent the limit of detection of organisms in the liver and spleen, respectively.

DISCUSSION

Although a number of earlier studies (1, 13–16, 19, 21, 26), including one conducted with the IgG2a MAb produced by clone 34 (16), have affirmed a protective role of antibodies against *B. abortus* in the murine model system, the data have demonstrated statistically significant reduction, rather than prevention, of infection. The present study was performed to determine whether antibodies could actually prevent the establishment of infection. Experimental conditions were set, on the one hand, to favor that outcome, and on the other, to provide as rigorous as possible a test of the question. Therefore, the infecting strain was preincubated with antibodies and the challenge dose was reduced in most experiments to one just large enough to establish a chronic infection. Concomitantly, we increased the sensitivity of our assay system 10-fold, so that "infection-free" status meant the presence of fewer than 13 brucellae in the entire spleen and fewer than 39 in the entire liver. Experiments were carried out for at least 4 and up to 6 weeks to ensure that animals judged free of infection at early intervals did not possess niduses of infection that would later produce detectable numbers of bacteria. The results of this study provide

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TABLE 2. Numbers of *B. abortus* organisms in spleens and antibody levels for LPS in serum of mice 6 weeks after challenge with *B. abortus* opsonized with an IgG2a MAb[a]

Amt of MAb per mouse (μg)	Mouse no.	No. of brucellae in spleen (10^2)	Antibody level (slope × 10^3)[b]
50	1	–[c]	16
	2	–	134
	3	–	12
	4	–	22
	5	–	37
10	6	–	16
	7	–	9
	8	–	8
	9	–	11
	10	–	10
1	11	–	8
	12	–	7
	13	–	68
	14	–	87
	15	–	154
0	16	–	49
	17	1,600	178
	18	6,200	27
	19	18	181
	20	1	34

[a] Infection was performed intravenously with approximately 100 CFU of *B. abortus* 2308 following incubation for 30 min at 37°C with graded quantities of MAb.

[b] Slope values from kinetics-based enzyme-linked immunosorbent assay with *B. abortus* S-LPS (f6) as antigen. Slope values for normal mice were approximately 10.

[c] –, ≤12 organisms per spleen. All mice with a culture-negative spleen also had a culture-negative liver.

TABLE 3. Numbers of *B. abortus* in spleens and antibody levels for LPS in serum of mice 6 weeks after challenge with *B. abortus* opsonized with a polyclonal antiserum[a]

Antiserum dilution	Mouse no.	No. of brucellae in spleen (10^2)	Antibody level (slope × 10^3)[b]
1:5	1	–[c]	30
	2	–	28
	3	–	20
	4	–	38
	5	0.4	42
1:50	6	–	13
	7	–	15
	8	–	10
	9	–	13
	10	–	12
1:500	11	–	10
	12	–	12
	13	–	11
	14	110	191
	15	–	15
0	16	80	230
	17	13	195
	18	0.2	60
	19	1	98
	20	19	182
	21	1	221
	22	2	150

[a] Infection was performed intravenously with approximately 100 CFU of *B. abortus* 2308 following incubation for 30 min at 37°C with graded dilutions of antiserum.

[b] Slope values from kinetics-based enzyme-linked immunosorbent assay with *B. abortus* S-LPS (f6) as antigen. Slope values for normal mice were approximately 10.

[c] –, ≤12 organisms per spleen. All mice with a culture-negative spleen also had a culture-negative liver.

strong evidence that antibodies specific for the O polysaccharide can prevent the establishment of infection with *B. abortus* in a majority of normal mice, although success in this endeavor was limited to the IgG2a MAb (Fig. 1). At 6 weeks p.i., antibody responses were negative in most mice treated with the IgG2a MAb, as well as in a majority of those infected with serum-opsonized bacteria (Table 2). Since the latter group of mice would almost certainly have been culture positive at 1 and 2 weeks p.i. (Fig. 3a), a negative antibody response at 6 weeks p.i. must be interpreted to signify either a lack of prior infection or the establishment of a transient infection which was eliminated by 6 weeks p.i. The possibility cannot be excluded that the passively transferred antibody itself inhibited the formation of antibody (17, 22, 27). However, the fact that negative responses were more consistent in the group receiving a smaller quantity of MAb (10 μg) (Table 2) suggests that antibody-mediated immunosuppression was not the predominant factor.

Infection was prevented or rapidly terminated in the majority of mice (73%) given a challenge dose of 500 CFU preopsonized with 50 μg of the IgG2a MAb (Fig. 2a), but all animals became infected when the dose was increased to 5,000 organisms (Fig. 2d). This may have been due to an inadequate concentration of MAb, although with a challenge dose of 100 CFU there was no evidence for a dose-response effect with two of the three opsonins used, and the effectiveness of the intermediate concentrations of the IgG2a MAb (Fig. 1; Table 2) and whole serum (Fig. 3; Table 3) appeared to have equaled or exceeded that of the highest concentrations.

In contrast to the effects of the IgG2a MAb, opsonization with the IgG1 MAb (Table 1) or with whole serum (Fig. 3a) failed to prevent infection with *B. abortus*, although pretreatment with whole serum caused a markedly accelerated elimination of infection in most mice, which was evident at 4 and 6 weeks p.i. (Fig. 3a). The basis for these differences has not yet been established. It should be emphasized that few direct comparisons among these preparations can be made in the concentrations, affinities, or fine specificities of the antibodies which they contained. Although all of the antibody preparations demonstrated opsonic activity in vivo, and killing of *B. abortus* followed similar kinetics in conventional medium (Fig. 4a), it is still probable that the IgG2a MAb functioned more effectively in vivo. In mice, IgG2a antibodies bind to the high-affinity type 1 Fc receptor (28) and are active in complement fixation (11). These properties, coupled with the high affinity of the clone 34 MAb (16), would have resulted in rapid and stable binding of antibody to both bacterial and phagocyte surfaces, resulting in efficient opsonization and maximal Fc receptor-mediated triggering of phagocyte function (4, 8). Although killed *B. abortus* has been reported to cause preferential synthesis of IgG2a antibody through stimulation of gamma interferon secretion (7), antibodies of the IgG2a isotype form a very low proportion of O-polysaccharide-specific antibodies in mice vaccinated with *B. abortus* endotoxin or LPS (10). This is also reflected in the low frequency of O-polysaccharide-specific MAbs of the IgG2a isotype (L. G. Adams, unpublished data). A low level of IgG2a antibodies in the serum pool used in this study may afford a partial explanation for

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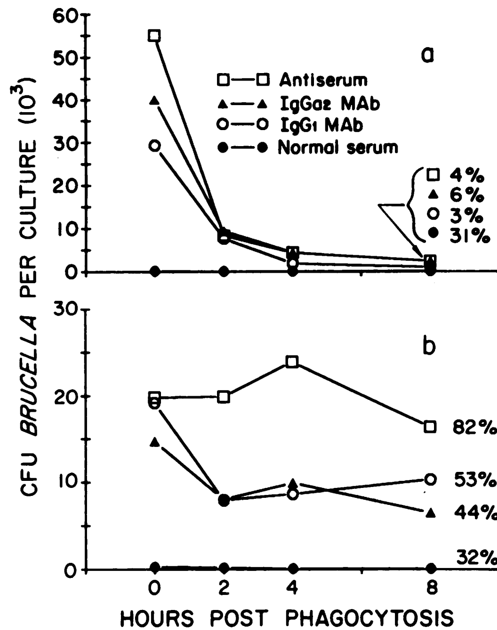

FIG. 4. Phagocytosis and killing of *B. abortus* 2308 by unelicited mouse peritoneal macrophages. Bacterial cells were preincubated with an IgG2a MAb (20 μg/ml), an IgG1 MAb (1:10), a heat-inactivated polyclonal antiserum pool (1:3,200), or normal heat-inactivated mouse serum (1:3,200). Tests were performed with conventional reagents (a) or with endotoxin-free reagents and pyrogen-free glassware (b). Percentages refer to the percentage of intracellular bacteria surviving for 8 h after phagocytosis.

the incapacity of this preparation to prevent infection (Fig. 3a). Enhancement of phagocytosis and intracellular killing probably constitute the principal functions of antibody in mediating protection against *B. abortus*, in particular if the inhibition of complement-mediated killing by antibodies of IgG isotypes observed in cattle (5) extends to other species. Variations among mice in isotypes of O antibodies mediating crucial protective functions may have an important bearing on the magnitude of protection afforded by vaccination with strain 19 or on the rate of resolution of a primary infection, which shows no correlation between overall levels of antibodies and bacterial counts in organs (30) (Table 2).

The in vitro assays were performed to ensure that each of the antibody preparations opsonized *B. abortus* and to determine whether virulent *B. abortus* would be killed within nonactivated macrophages in which cell function had been stimulated following Fc receptor binding (4, 8). In accord with prior reports (8, 31), phagocytosis of smooth *B. abortus* was negligible in the absence of opsonins (Fig. 4). The data (Fig. 4) indicate that opsonized *B. abortus* is effectively killed only by macrophages which have been primed. Initial attempts to enhance the killing of brucellae in endotoxin-free medium by pretreating monocytes with LPS of *B. abortus* or *Escherichia coli* O111:B4 proved inconclusive (C. G. Santisteban and A. J. Winter, unpublished data). Nevertheless, the most probable source of monocyte priming was LPS, for which this property is well documented (2, 9, 18) and can be initiated within 1 h (18).

It seems reasonable to believe that in the cow, as well as in the mouse, O polysaccharide-specific antibodies could play an important role in protection and that the induction of maximum protection in cattle may not be possible with vaccines which fail to stimulate O antibodies of the appropriate isotypes. If this proves true, a solution would be required for the interference caused by O antibodies in the interpretation of serodiagnostic tests (25). Since the O polysaccharide of *B. abortus* contains more than one epitope (3, 6), one strategy might be a dissection of the epitopes into those which are essential for protection and those which are not. A vaccine could be constructed to incorporate only an essential epitope, thus allowing ready distinction of cattle which had been vaccinated from those responding to infection with field strains of *B. abortus*.

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