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Comparison of Living and Nonliving Vaccines for *Brucella abortus* in BALB/c Mice

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The BALB/c mouse was selected as a model for infection with *Brucella abortus* on the basis of protracted nonclinical infection produced by strain 2308, virulent for cattle, and relatively rapid clearance of strain 19, an attenuated strain used to vaccinate cattle. Protection in mice vaccinated with strain 19 was compared with that obtained with nonliving vaccines at early (1 week) and later (4 weeks) intervals after challenge with strain 2308 and assessed by enumeration of *B. abortus* organisms in the spleen. Mice challenged 4 weeks after vaccination with strain 19 exhibited significant protection at 1 and 4 weeks postinfection (p.i.), with an increased magnitude of protection at the later time. When challenged 6 weeks after vaccination with strain 19, the level of protection diminished between 1 and 4 weeks p.i. and at the later time was not always significantly different from controls. Mice immunized 4 weeks earlier with nonliving vaccines in mineral oil with trehalose dimycolate (TDM) and muramyl dipeptide (MDP) demonstrated patterns of protection similar to those obtained following the 6 week vaccination-challenge interval with strain 19. Vaccination with cell envelopes derived from strain 2308 produced equivalent protection at 1 week p.i. whether administered in phosphate-buffered saline, incomplete Freund adjuvant, or the TDM and MDP adjuvant. Equivalent protection also followed vaccination with strain 2308 killed whole cells, cell envelopes, or outer membrane proteins in phosphate-buffered saline or in the TDM and MDP adjuvant. The TDM and MDP adjuvant alone induced nonspecific resistance, which peaked at 1 day p.i. and was still present at 1 week p.i., although by this time its magnitude was significantly less than the protection induced by antigen combined with the adjuvant. These data, together with the results of antibody assays and passive and adoptive transfer studies, suggested that protection at 1 week p.i. could be accounted for largely by an effect of O antibodies, with T cell-mediated immune responses having a subsidiary role.

Brucella abortus causes bovine brucellosis, which is characterized principally by abortion and chronic infection within lymph nodes and in the mammary gland (34). The current understanding of *B. abortus* as a parasite and of protective immunity in brucellosis is still based largely on studies in mice, for which *B. abortus* is a facultative intracellular parasite (7, 17) against which protection is mediated by T lymphocytes (7). However, there is also evidence that antibodies confer immunity (3, 18, 22, 27, 30, 35). The mouse has also been used as a model to evaluate *Brucella* vaccines (3, 5, 35).

We hypothesized that protective immunity to brucellosis could be achieved in cattle with a nonliving vaccine, provided an adjuvant was employed which would induce cell-mediated immunity (CMI) as well as antibody responses (38). For this purpose we tested in cattle vaccines combined with trehalose dimycolate (TDM) and muramyl dipeptide (MDP) (38), adjuvants which are effective in inducing CMI responses (for reviews, see references 1 and 16). We also sought to develop a model system to screen vaccines to permit selection of those most promising for protection trials in cattle. In the present study strain 19 was used as a standard vaccine in mice against which to compare the immunity conferred by nonliving vaccines given without adjuvant, with incomplete Freund adjuvant (IFA), and with TDM and MDP. Adoptive and passive transfer studies were performed to evaluate the contribution to protection of cell-mediated and humoral immune responses.

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MATERIALS AND METHODS

Mice. BALB/cByJ and C57BL/10 SnJ mice were obtained from Jackson Laboratories, Bar Harbor, Maine, 1 week before use.

Bacterial strains for vaccination and challenge. *B. abortus* 19 (Biologics Division, U.S. Dept. of Agriculture) was used as a vaccine, and strain 2308 (36) was used as the challenge strain. Stock cultures were prepared as described previously (36) and held at −70°C. Stock cultures were grown for 48 h on Schaedler blood agar plates (36), and the cells, suspended in sterile phosphate-buffered saline (PBS), were adjusted turbidimetrically to the desired concentration. The exact dose was calculated retrospectively by viable counts.

Antigens for nonliving vaccines. Killed whole cells, cell envelopes, and outer membrane proteins were prepared from strain 2308 as described previously (38).

Adjuvants. TDM extracted from *Mycobacterium bovis* was purchased from Choay Chimie Reactifs (Paris, France), and a derivative of MDP, *N*-acetylmuramyl-L-α-aminobutyryl-D-isoglutamine, was a gift from G. H. Jones, Syntex Reseach, Palo Alto, Calif. IFA was purchased from Sigma Chemical Co., St. Louis, Mo.

Preparation and administration of nonliving vaccines. Vaccines containing TDM and MDP were prepared as described by Winter et al. (38). In most experiments each mouse received 30 μg of antigen, 10 μg of MDP, 20 μg of TDM, and 2.4 μl of mineral oil in 0.2 ml of a Tween-water solution. When lower antigen doses were used, TDM was decreased to maintain a ratio of 1 part TDM to 2 parts remaining dry matter, and oil was adjusted to maintain 40 μg/mg of total

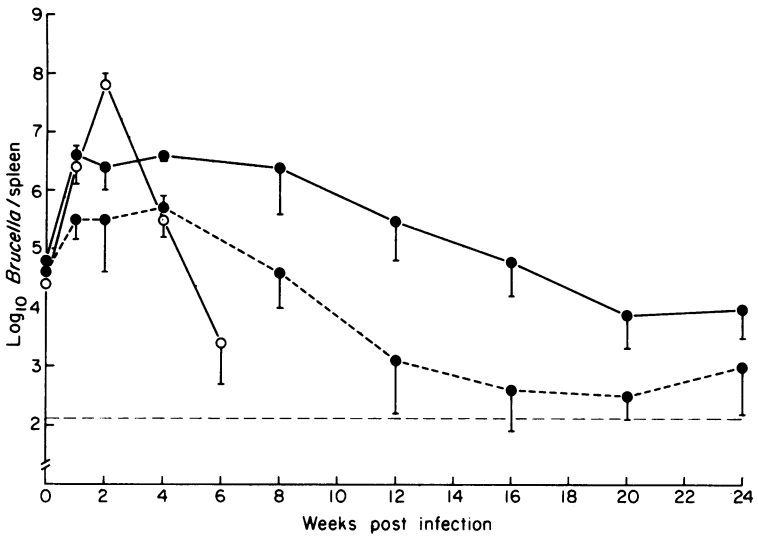

FIG. 1. Course of infection by *B. abortus* 2308 (●) and 19 (○) in spleens of BALB/c (——) and C57BL/10 (– – –) mice. Bars indicate standard deviations. Dashed line at $\log_{10}$ 2.12 represents the limit of detection of organisms in the spleen.

dry weight. Vaccines in IFA contained equal parts oil and antigen solution in PBS.

T lymphocyte enrichment. Single-cell suspensions from spleens were prepared by homogenizing the organs with a glass tissue grinder in 1 ml of Hanks balanced salt solution supplemented with 2% fetal calf serum (Difco Laboratories, Detroit, Mich.). Pooled cells were separated on nylon wool columns by the method of Greaves et al. (12). Cell viability was estimated by trypan blue exclusion. Nonadherent fractions were stained with fluorescein isothiocyanate-conjugated rabbit anti-mouse brain (Bionetics, Laboratory Products Division, Charleston, S.C.) and fluorescein isothiocyanate-conjugated $F(ab')_2$ goat anti-mouse immunoglobulin M (IgM) (Cappel Laboratories, Scientific Division, Malvern, Pa.). In the separate experiments the proportion of T cells ranged from 80 to 90%.

Preparation of sera for passive immunization. Mice were bled from the retroorbital sinus. Sera were pooled, heat inactivated at 56°C for 30 min, diluted 1:5 with PBS, filter sterilized, and held frozen at −20°C until use.

Experimental design. Four types of experiments were performed.

(i) Time course of infection. Ten-week-old mice were fasted overnight and infected intraperitoneally (i.p.) with *B. abortus* in 0.1 ml of PBS. At selected times postinfection (p.i.) groups of five mice were killed by cervical dislocation. Spleens were homogenized in 10 ml of PBS, serially diluted, and plated in triplicate.

(ii) Vaccination followed by challenge. Six-week-old mice were injected with vaccines or control preparations. At various intervals thereafter, they were infected with strain 2308. Nonliving vaccines were given intramuscularly (i.m.) and living vaccines or challenge infections i.p. unless otherwise noted. At times p.i. ranging from 1 day to 4 weeks, groups of five mice per treatment were killed and spleen counts were performed. In experiments with strain 19, counts were also done on plates with 0.01% erythritol to distinguish strains 19 and 2308 (2).

(iii) Adoptive transfer with lymphocytes. Donor mice were vaccinated at the age of 6 weeks. At selected intervals thereafter, groups of vaccinated and control mice were bled, and T lymphocyte-enriched suspensions were prepared from the spleens. Groups of five 10-week-old recipients were injected intravenously with 3×10^7 viable lymphocytes per mouse in 0.1 ml of PBS containing 1% fetal calf serum. Two hours later recipient mice were challenged i.p. with strain 2308, and *B. abortus* cells were enumerated in their spleens 1 and 4 weeks later.

(iv) Passive transfer with serum. Groups of five 10-week-old mice were injected i.p. with 0.5 ml of 1:5 dilutions of serum derived from the same donor mice used for adoptive transfer experiments. Challenge infections were performed 3 to 4 h later, and spleen counts were made 1 and 4 weeks p.i.

Immunoassay. For the enzyme-linked immunosorbent assay, donor serum pools were titrated against purified porin and group 3 proteins of the rough *B. abortus* strain 45/20 (36) and the partially purified lipopolysaccharide fraction (f6) of strain 2308 (29). Each antigen was used at 500 ng per well. Proteins from strain 45/20 are highly cross-reactive with those of strain 2308 (37) and were used because proteins derived from smooth strains contain O antigen on the adherent lipopolysaccharide (36). The highest dilution with an absorbance 0.2 units greater than the control was taken as the titer.

Statistical methods. A mean value for each spleen count was obtained by averaging the triplicate values following log conversion. The lowest number of colonies detectable per spleen was estimated at 133, so spleens in which no colonies were detected were assigned a number of 132 ($\log_{10}$ = 2.12) for purposes of calculation. Statistical analyses were performed with a Studentized range test (33).

RESULTS

Growth patterns of *B. abortus*. BALB/c mice were infected with graded quantities of strain 2308 decreasing in fivefold increments from the highest dose of 10^6 CFU. In several experiments, spleen counts in groups receiving 5×10^4 organisms ranged from 3×10^6 to 6×10^6 after 1 week and remained at this level or decreased slightly by 2 weeks p.i. Larger infecting doses did not produce higher counts, whereas in mice receiving doses lower than 5×10^4, spleen

TABLE 1. Effects of vaccination with graded quantities of *B. abortus* 19 against challenge with *B. abortus* 2308[a]

Vaccination (CFU)	Log strain 2308 cells in spleens (mean ± SD)	
	1 wk p.i.	4 wk p.i.
None	6.20 ± 0.85	6.25 ± 0.26
Strain 19		
2.9×10^1	3.68 ± 1.07*	5.50 ± 0.70
2.9×10^2	4.81 ± 1.90*	5.84 ± 0.86
2.9×10^3	4.27 ± 1.00*	4.85 ± 0.89
2.9×10^4	2.91 ± 0.97*	4.98 ± 1.01

[a] Vaccination was performed i.p. 6 weeks prior to challenge i.p. with approximately 5×10^4 CFU of strain 2308. Plates contained 0.01% erythritol. *, $P < 0.05$ compared with untreated control group. Analysis of pooled data.

counts were progressively lower at 1 week p.i. In a time course experiment over 16 weeks, splenic infection in BALB/c mice reached a plateau until 8 weeks p.i. and then declined gradually. This was confirmed in a 24-week trial in which substantial numbers of *B. abortus* were still present when the experiment was terminated (Fig. 1). In C57BL/10 mice the infection followed a similar pattern, but the plateau phase was shorter and the number of organisms recovered from spleens was at most time periods significantly below that recovered from BALB/c mouse spleens (Fig. 1). In 8 of 15 C57BL/10 mice tested at or after 16 weeks p.i., there were no detectable organisms.

The growth of strain 19 was tested in BALB/c mice infected with 3.7×10^4 (Fig. 1) or 3.8×10^5 CFU. In both groups infections peaked at 2 weeks p.i., with splenic counts approximately 1 log greater than those attained with strain 2308 (Fig. 1). Infection declined sharply thereafter, and spleen counts by 6 weeks were below those in BALB/c mice infected with strain 2308 at 24 weeks p.i. (Fig. 1). A repetition of this experiment revealed the same pattern of growth.

BALB/c mice were selected for vaccine trials because of their greater susceptibility to infection with strain 2308. A dose of 5×10^4 organisms of strain 2308 was used to provide the most rigorous challenge, and protection was assessed at

TABLE 2. Effects of various vaccination-challenge intervals in mice vaccinated with *B. abortus* 19

Vaccination[a]	Challenge[b]	Vaccination-challenge interval (wk)	Log *B. abortus* cells in spleens (mean ± SD)[c]	
			1 wk p.i.	4 wk p.i.
No	Yes		6.70 ± 0.16[d]	ND[e]
Yes	Yes	2	5.88 ± 0.80[d]*	6.22 ± 1.02
Yes	No		4.80 ± 1.75[f]	4.46 ± 1.26
No	Yes		6.58 ± 0.14	6.37 ± 0.68
Yes	Yes	4	4.93 ± 0.80**	3.86 ± 0.55**
Yes	No		3.50 ± 1.63	3.11 ± 0.80
No	Yes		6.71 ± 0.15	6.60 ± 0.41
Yes	Yes	6	4.27 ± 0.36**	5.45 ± 0.67*
Yes	No		2.88 ± 1.04	2.12[g]

[a] Approximately 5×10^4 cells of strain 19, subcutaneously.
[b] Approximately 5×10^4 cells of strain 2308, intravenously.
[c] **, $P < 0.01$ compared with unvaccinated control group; *, $P < 0.05$ compared with unvaccinated control group.
[d] Plates contained 0.01% erythritol, detecting strain 2308.
[e] ND, Not done. Mice died from accidental causes during holding period.
[f] Plates without erythritol, detecting strain 19.
[g] No *B. abortus* organisms were detected in any spleens from this group.

TABLE 3. Effects of vaccination with cell envelopes of *B. abortus* 2308 with and without adjuvants[a]

Vaccine[b]	Adjuvant	Log strain 2308 cells in spleens (mean ± SD)	
		1 wk p.i.	4 wk p.i.
None	None	6.44 ± 0.23	6.16 ± 0.28
CE	None (PBS)	3.53 ± 0.43**	5.24 ± 0.58
CE	TDM-MDP	3.16 ± 0.33**	4.99 ± 1.0*
CE	IFA	3.03 ± 0.61**	5.98 ± 0.64
None	TDM-MDP	5.09 ± 0.14**	6.62 ± 0.24
None	IFA	6.49 ± 0.26	6.47 ± 0.34

[a] Vaccinations were performed i.m. 4 weeks prior to challenge i.p. with approximately 5×10^4 cells of strain 2308. **, $P < 0.01$ compared with untreated control group; *, $P < 0.05$ compared with untreated control group.
[b] CE, Cell envelope preparation.

an early time period (1 week p.i.) as well as a later time during the plateau phase (4 weeks p.i.).

Vaccination with strain 19. Mice were vaccinated with a range of doses of strain 19. Challenge infections were performed 6 weeks later so that at challenge the number of residual strain 19 cells in spleens would be minimal (Fig. 1). On the basis of differential counts on plates with and without erythritol, the number of strain 19 cells was negligible in all groups except at 1 week p.i. in the group receiving the highest vaccine dose (data not shown). Vaccination with strain 19 caused a reduction in strain 2308 (Table 1) which did not differ significantly among the vaccinated groups within either time period. The number of organisms in the vaccinated groups was significantly lower than in the controls at 1 week ($P < 0.05$) but not at 4 weeks p.i.

The clearance of strain 19 from spleens of BALB/c mice progressed rapidly after 2 weeks (Fig. 1), indicating the development by this time of an effective immune response. We therefore assessed protection against strain 2308 at earlier vaccination-challenge intervals. At 2 weeks after vaccination mice manifested less than 1 log of protection at 1 week p.i. ($P < 0.05$), whereas with a vaccination-challenge interval of 4 weeks, significant protection ($P < 0.01$) was evident at both 1 and 4 weeks p.i., and protection at the 4-week time point (2.5 logs) exceeded that at 1 week (1.6 logs) (Table 2). At a vaccination-challenge interval of 6 weeks, protection at 1 week p.i. (2.4 logs; $P < 0.01$) was greater than at 4 weeks (1.2 logs; $P < 0.05$) (Table 2), in accord with the prior experiment (Table 1). The improve-

TABLE 4. Effects of vaccination with different doses of cell envelopes of *B. abortus* 2308[a]

Vaccine[b] (μg)	Adjuvant	Log strain 2308 cells in spleen (mean ± SD)	
		1 wk p.i.	4 wk p.i.
None	None	6.54 ± 0.32	6.53 ± 0.44
CE (30)	TDM-MDP	4.33 ± 0.38**	6.83 ± 0.32
None	TDM-MDP[c]	5.57 ± 0.73	6.77 ± 0.18
CE (3)	TDM-MDP	3.84 ± 0.21**	6.68 ± 0.21
None	TDM-MDP[d]	5.14 ± 0.42*	6.45 ± 0.60
CE (0.3)	TDM-MDP	4.0 ± 0.56**	6.68 ± 0.70
None	TDM-MDP[e]	4.97 ± 0.79**	6.65 ± 0.49

[a] Vaccinations performed i.m. 4 weeks prior to challenge i.p. with approximately 5×10^4 strain 2308. **, $P < 0.01$ compared with untreated control group; *, $P < 0.05$ compared with untreated control group.
[b] CE, Cell envelopes of strain 2308.
[c] Corresponding to quantity used with 30 μg of CE.
[d] Corresponding to quantity used with 3 μg of CE.
[e] Corresponding to quantity used with 0.3 μg of CE.

TABLE 5. Magnitude of nonspecific resistance due to TDM-MDP adjuvant over time[a]

Vaccine	Adjuvant	Log strain 2308 cells in spleens (mean ± SD)		
		1 day p.i.	1 wk p.i.	4 wk p.i.
None	None	4.66 ± 0.02	6.70 ± 0.18	6.52 ± 0.54
CE[b]	TDM-MDP	2.37 ± 0.43**	3.12 ± 0.57**	5.73 ± 0.53
None	TDM-MDP	2.45 ± 0.31**	4.62 ± 0.42**	6.19 ± 0.62

[a] Vaccination was performed i.m. 4 weeks prior to challenge i.p. with approximately 5×10^4 cells of strain 2308. **, $P < 0.01$ compared with untreated control group; for other comparisons, see the text.
[b] 30 μg of cell envelopes of strain 2308.

ment in protection between weeks 1 and 4 p.i. in mice challenged 4 weeks after vaccination was confirmed in a subsequent experiment (1 week: 0.81 logs, $P < 0.01$; 4 weeks: 1.99 logs, $P < 0.01$). Results obtained with subcutaneous vaccination and intravenous challenge have been found not to differ in this system from those obtained with the i.p. route (A. J. Winter and G. E. Rowe, unpublished data).

Vaccination with nonliving antigens. Mice were immunized with 30 μg of cell envelopes in PBS and in TDM-MDP or IFA adjuvants, and *B. abortus* organisms were enumerated in the spleens at 1 and 4 weeks p.i. (Table 3). All vaccines conferred about 3 l ogs of protection at 1 week p.i. ($P < 0.01$), and there was no difference among them. The TDM-MDP adjuvant itself caused a significant reduction in infection at 1 week pi ($P < 0.01$), although counts in this control group were significantly greater than those in the group receiving antigen with TDM and MDP ($P < 0.01$). At 4 weeks p.i. spleen counts were significantly decreased ($P < 0.05$) only in the group vaccinated with cell envelopes in TDM-MDP. IFA alone produced no effect (Table 3).

Two dose-response tests performed in mice immunized with 30, 3, or 0.3 μg of cell envelopes in TDM-MDP adjuvant yielded the same results. Significant protection was observed only at 1 week p.i. ($P < 0.01$), but no differences occurred among the vaccinated groups (Table 4). The nonspecific protective effect of adjuvant was again manifested (Table 4). Counts in groups receiving 30 or 3 μg of antigen were significantly below those in the corresponding adjuvant control groups ($P < 0.05$). In another experiment this nonspecific effect was found to be most pronounced at 1 day p.i., when counts in mice given adjuvant alone were equivalent to those in mice vaccinated with antigen and adjuvant (Table 5). By 7 days p.i. the group receiving antigen had lower counts than that receiving adjuvant alone ($P < 0.01$) (Table 5). Our data on nonspecific resistance with these adjuvants are consistent with the observations of others (6, 15, 19, 26).

Killed whole cells and outer membrane proteins were compared with cell envelopes as vaccines (Table 6). The three vaccines did not differ from each other in protective value. All produced 2 to 3 logs of protection ($P < 0.01$) at 1 week p.i. and no effect at the later time. In this experiment counts in the adjuvant control group at 1 week p.i. were not significantly reduced. In two additional experiments comparable protection was obtained by vaccination with cell envelopes or outer membrane proteins. At 1 week p.i. equivalent protection ($P < 0.01$) was again achieved with these antigens in PBS or TDM-MDP adjuvant, whereas at 4 weeks p.i. significant protection ($P < 0.05$) occurred only with antigens in adjuvant.

Adoptive and passive transfer studies. Donor groups were immunized with strain 19 (7.8×10^2 CFU per mouse), 30 μg of cell envelope in TDM and MDP adjuvant, or 30 μg of cell envelope in IFA. A reduction of counts was demonstrated at 1 week p.i. in each recipient group receiving immune cells (Table 7), although in no instance were counts significantly below those in untreated controls. However, in groups receiving cells from donors vaccinated with strain 19 or cell envelopes in IFA, spleen counts were about 1 log ($P < 0.01$) and 0.7 log ($P < 0.05$) lower, respectively, than in the corresponding groups receiving normal cells. In recipient groups injected with pooled sera from the same donor groups, spleen counts at 1 week p.i. did not differ from each other but were 2 to 3 logs lower than in the untreated group ($P < 0.01$) and the group that received normal serum ($P < 0.01$) (Table 8). Increased titers of O antibodies were detected in the serum pools, in particular that from the group immunized with strain 19 (Table 8). Titers of antibodies to porin or group 3 antigens were generally lower and did not differ appreciably from those in normal mouse serum (Table 8).

The experiments were repeated, except that spleen counts were performed 4 weeks after transfer of lymphocytes or antisera. No significant differences in counts were observed in any group as a consequence of adoptive or passive transfer.

Two more experiments were performed to compare the protective capacity of splenic T cells at different intervals after vaccination with strain 19. In both experiments T cells taken 6 weeks after vaccination conferred low (<0.5 log compared with uninjected controls) but significant ($P < 0.05$) levels of protection, whereas cells taken 2 weeks after vaccination did not.

DISCUSSION

The kinetics of infection with strain 19 in BALB/c mice (Fig. 1) were very similar to those described by Ho and Cheers (13). In contrast, strain 2308 produced a very protracted infection in BALB/c mice, whereas in C57BL/10 mice the level of infection was consistently lower (Fig. 1). The development by BALB/c mice of chronic infections with *B. abortus* without overt disease corresponds to chronic

TABLE 6. Effect of vaccination with different nonliving antigens derived from *B. abortus* 2308[a]

Vaccine (μg)	Adjuvant	Log strain 2308 cells in spleens (mean ± SD)	
		1 wk p.i.	4 wk p.i.
None	None	6.18 ± 0.27	6.62 ± 0.44
Killed whole cells (30)	TDM-MDP	3.49 ± 0.77**	6.56 ± 0.47
Cell envelopes (30)	TDM-MDP	3.77 ± 0.28**	6.23 ± 0.51
Outer membrane proteins (30)	TDM-MDP	3.50 ± 0.50**	6.64 ± 0.19
None	TDM-MDP	5.33 ± 0.78	6.42 ± 0.65

[a] Vaccination was performed i.m. 4 weeks prior to challenge i.p. with approximately 5×10^4 cells of strain 2308. **, $P < 0.01$ compared with untreated control group.

TABLE 7. Effect of adoptive transfer of cells from donors immunized with living or nonliving *B. abortus* vaccines

Expt no.	Treatment of recipients[a]	Log strain 2308 cells in spleens (mean ± SD) at 1 wk p.i.[b]
1	None	6.03 ± 0.53
	Normal cells	6.42 ± 0.46
	Immune cells	5.45 ± 0.36**
2	None	6.46 ± 0.29
	Normal cells	6.13 ± 0.41
	Immune cells	5.95 ± 0.46
3	None	6.12 ± 0.17
	Normal cells	6.32 ± 0.35
	Immune cells	5.62 ± 0.60*

[a] Cells were administered intravenously 2 h prior to challenge i.p. with approximately 5×10^4 cells of strain 2308. Normal cells, 3×10^7 T-enriched lymphocytes from spleens of normal mice. Immune cells, 3×10^7 T-enriched lymphocytes from spleens of mice vaccinated i.p. 6 weeks earlier with strain 19 (experiment 1), mice vaccinated i.m. 4 weeks earlier with 30 μg of cell envelopes in TDM-MDP adjuvant (experiment 2), or mice vaccinated i.m. 4 weeks earlier with 30 μg of cell envelopes in IFA (experiment 3).

[b] **, $P < 0.01$ compared with group receiving normal cells; *, $P < 0.05$ compared with group receiving normal cells.

brucellosis in cattle. The relatively more rapid clearance of strain 19 than of strain 2308 also corresponds to the relative virulence of these strains in cattle (34), and in these respects indicates that the BALB/c mouse might serve as a useful model for brucellosis of cattle and for evaluating vaccines intended for cattle.

An effective vaccine must provide sustained protection with elimination of the challenge infection. The nonliving vaccines evaluated here produced substantial protection at 1 week p.i., but as a rule protection by 4 weeks had waned, and in the majority of experiments, was no longer statistically significant. In this respect strain 19 tested at 2 or 6 weeks after vaccination was no more effective than nonliving vaccines. In contrast, at a vaccination-to-challenge interval of 4 weeks, mice vaccinated with strain 19 demonstrated increased protection between 1 and 4 weeks p.i. Experiments are in progress to determine whether in these mice the challenge infection is progressively eliminated. In almost all of the studies reported by Plommet and his co-workers protection was assessed at a short interval p.i. (15 days) (4, 5, 9). However, long-term protection with elimination of infection has been reported in outbred mice vaccinated with PG (31), an antigen containing peptidoglycan, lipopolysaccharide, and denatured outer membrane proteins (9, 10). This difference may be due to variations in our test systems. Recent work in our laboratory has demonstrated that in BALB/c mice challenged with strain 2308 protection provided by vaccination with PG derived from strain 2308 was no better than that provided by native cell envelopes (A. J. Winter and G. E. Rowe, unpublished data).

A unifying feature among facultative intracellular parasites is the crucial role of CMI in protective immunity. However, this group of pathogens, exemplified by *Listeria monocytogenes* (21, 24), *Francisella tularensis* (14), *Mycobacterium tuberculosis* (23, 25), and *Salmonella typhimurium* (11), produces a spectrum of diseases which vary in chronicity, in the magnitude of protection developed in the host, and in the capacity of antibodies alone to mediate some measure of protective immunity. *B. abortus* shares selected properties of these pathogens. In agreement with the results of others (3, 18, 27, 30, 35), substantial levels of protective immunity could be conferred by antibodies alone (Table 8). In the study reported here, protection by antibodies was not demonstrable 4 weeks after challenge, but in passive transfer experiments with monoclonal O antibodies, significant levels of protection occurred at 1 and 4 weeks p.i. in both spleens and livers (22). Adoptive transfer experiments in mice infected with *B. abortus* have revealed more limited protection (28, 32), consistent with our data. In our experiments, spleen counts were decreased in groups that received immune cells, but differences were not significant unless comparisons were made between groups receiving immune cells and those receiving normal cells (Table 7). Normal cell controls were employed by both Pavlov et al. (28) and Plommet et al. (32). Although the reason is unclear, it has been observed in other systems that bacterial counts in tissues of mice receiving normal lymphocytes generally exceed those in animals receiving no cells (D. D. McGregor, personal communication). It is unclear why protection in recipients of cells from donors immunized with IFA was better than in those given cells from donors immunized with TDM-MDP adjuvant. One possibility is that the peptidoglycan in the cell envelope antigen sufficed to stimulate a CMI response and thus obviated the advantage of using exogenous MDP. In this respect it is notable that the adoptive transfers performed by Plommet et al. (32) used cells from donors vaccinated with PG without addition of adjuvants.

In mice vaccinated with strain 19, T cells capable of conferring immunity to strain 2308 did not appear in highest concentration in the spleen at the time that there was a sharp decrease in that organ of strain 19 organisms. These findings are in contrast to those with *L. monocytogenes* (20) and *F. tularensis* (14) but are in accord with the relative capacity of mice vaccinated with strain 19 to clear infection with strain

TABLE 8. Effect of passive transfer of sera from donors immunized with living or nonliving *B. abortus* vaccines

Treatment[a]	Antibody titer of transferred serum against:			Log strain 2308 cells in spleens (mean ± SD) at 1 wk p.i.[b]
	O antigen	Porin	Group 3 protein	
None				6.17 ± 0.59
Normal serum	<25	100	25	5.95 ± 0.57
Immune serum from donor vaccinated with:				
Strain 19 live cells	800	25	50	2.90 ± 0.71**
Strain 2308 cell envelopes, in TDM-MDP adjuvant	100	50	50	3.58 ± 0.37**
Strain 2308 cell envelopes in IFA	200	100	<25	3.09 ± 0.18**

[a] Sera were administered i.p. 3 to 4 h prior to challenge i.p. with approximately 5×10^4 cells of strain 2308. Immune sera were derived from the same donor groups used for adoptive transfer experiments (Table 7).

[b] $P < 0.01$ compared with either untreated or normal serum control groups.

2308 at vaccination-challenge intervals of 2 and 6 weeks (Table 2). These results leave open the question of the relative importance of immune T cells in the early clearance of strain 19 (Fig. 1). Based on our adoptive (Table 7) and passive (Table 8) (22) transfer studies, as well as the relative effectiveness of nonliving vaccines with and without adjuvants (Table 3), it may also be inferred that vaccinal immunity at 1 week p.i. was due in large measure to O antibodies, with a minor role for CMI and a contribution from nonspecific effects in groups given TDM-MDP adjuvant (Tables 3 through 6).

The problem remains of defining a nonliving vaccine which will confer effective immunity on a long-term basis. For this purpose a more precise understanding is required of the kinetics of development of the immune response, the relative roles in protection of humoral and CMI responses at progressive stages of infection, and the nature of the T effector cells which mediate protection. Furthermore, it is unlikely that this problem will be resolved until a more complete understanding is reached of the mechanisms by which virulent strains of *B. abortus* apparently evade the immune response of the host during the plateau phase (7, 8, 13).

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