INFECTION AND IMMUNITY, Aug. 1994, p. 3206–3212
0019-9567/94/$04.00+0

Vol. 62, No. 8

Immune and Pathologic Responses in Mice Infected with *Brucella abortus* 19, RB51, or 2308

MARK G. STEVENS,* STEVEN C. OLSEN, GEORGE W. PUGH, JR., AND MITCHELL V. PALMER

Brucellosis Research Unit, National Animal Disease Center, USDA Agricultural Research Service, Ames, Iowa 50010

Received 14 March 1994/Returned for modification 28 April 1994/Accepted 11 May 1994

Immune and pathologic responses were measured for 20 weeks after infection of mice with *Brucella abortus* 19, RB51, or 2308. Live bacteria and bacterial antigens of 19 and RB51 persisted in spleens for 10 and 4 weeks after infection, respectively, whereas 2308 bacteria and bacterial antigens persisted for at least 20 weeks. Small germinal centers and profound lymphoid depletion occurred in spleens of mice during the first 4 weeks of infection with strain 19 or 2308; however, mice infected with strain RB51 had much larger germinal centers but no lymphoid depletion. At 4 weeks, only spleen cells from RB51-infected mice proliferated when incubated with 2308 bacteria. Large germinal centers in the spleen and spleen cell proliferative responses to 2308 did not appear in strain 19-infected mice until 6 weeks or in strain 2308-infected mice until 10 weeks. Similar proliferative responses to 2308 occurred in mice infected with strain 19 or RB51 at 6 weeks and in mice infected with strain 19, RB51, or 2308 at 10 weeks. However, at 20 weeks, spleen cell proliferative responses to 2308 occurred in mice infected with strain 19 or 2308 but not in mice infected with strain RB51. Mice infected with strain RB51 had lower and less persistent antibody titers to 2308 than did mice infected with strain 19 or 2308. Collectively, these results indicate that RB51-infected mice have less persistent immune responses to 2308 than do mice infected with 19 or 2308. The shorter duration of the responses probably resulted because RB51 is considerably less pathogenic and is cleared more rapidly from mice than are 19 and 2308.

Brucella abortus is an intracellular bacterium that causes abortions in cattle (6) and chronic infections such as undulant fever, endocarditis, arthritis, and osteomyelitis in humans (27). Abortions in cattle are currently prevented by immunizing heifers with the *B. abortus* 19 vaccine (14). However, the strain 19 vaccine induces production of antibodies to the lipopolysaccharide (LPS) O side chain of *B. abortus*, which can be detected by conventional serologic tests used to diagnose brucellosis in cattle (11, 25). Consequently, vaccinating cattle with strain 19 complicates the interpretation of diagnostic serologic tests used to detect cattle that have been naturally infected with field strains of *B. abortus* (11).

B. abortus RB51 is a laboratory-derived rough mutant of the virulent strain 2308 of *B. abortus* (21). Strain RB51 resembles strains 19 and 2308 in that it contains the same outer membrane proteins (19, 21); however, it differs from the other two strains in that it lacks virtually all of the LPS O side chain (20, 21). Therefore, antibodies to the LPS O side chain of *B. abortus* are not induced in RB51-vaccinated cattle (4, 5, 21, 23). Cattle vaccinated with strain RB51 also have cell-mediated immune responses to strain 2308 (24) and are protected from abortions following challenge with virulent 2308 cells in a similar manner as occurs when cattle are vaccinated with strain 19 (5). These attributes suggest that strain RB51 might be better than strain 19 as a vaccine in cattle, because RB51 induces immunity without inducing serologic responses to LPS that are detected by diagnostic tests for brucellosis. Therefore, the RB51 vaccine may enable more efficient serologic identification and removal of cattle with brucellosis from vaccinated herds in the United States (23).

* Corresponding author. Mailing address: NADC, USDA, ARS, 2300 Dayton Ave., Ames, IA 50010. Phone: (515) 239-8313. Fax: (515) 239-8458.

Most of the current understanding of the pathogenesis and immunity of animal brucellosis has arisen from studies in mice. Studies in mice with strain RB51 have produced similar results to those seen in cattle. For example, RB51 is less virulent than 19 (26), and mice vaccinated with RB51 have enhanced resistance to infection with 2308 (21). However, the immune and pathologic responses that are induced by strain RB51 have not been characterized and compared with the responses induced by the parental strain 2308 or 19 currently used to vaccinate cattle. In this study, the onset and duration of the immune and pathologic responses in RB51-infected mice were compared with those in mice infected with strain 19 or 2308.

MATERIALS AND METHODS

***B. abortus* cultures.** Live suspensions of strains 2308, 19, and RB51 were prepared as described previously (24). Killed suspensions of strains 2308, 19, and RB51 were prepared by γ-irradiating 3×10^{11} CFU/ml of 0.01 M phosphate-buffered saline (PBS [pH 7.2]) with 1.4×10^6 rads. The killed bacteria were washed three times by centrifugation in PBS, aliquoted, and then stored in PBS at −70°C.

Infection of mice with *B. abortus* and collection of tissues. Female 8-week-old BALB/c mice were obtained from Charles River Breeding Laboratories Inc., Wilmington, Mass.; they were used in the experiments when they were 10 weeks old. The mice (10 per treatment group) were given intraperitoneal (i.p.) injections of 0.2 ml of a 0.15 M NaCl saline solution (controls) or 0.2 ml of saline containing approximately 10^5 CFU of strain 2308 or 10^7 CFU of either strain 19 or RB51. The precise number of CFU was determined retrospectively by viable plate counts and found to be 1.2×10^5 for 2308, 8.3×10^6 for 19, and 9.2×10^6 for RB51. Blood samples and spleens were obtained from age-matched noninfected control mice and from infected mice at 2, 4, 6, 10, or 20 weeks after infection.

TABLE 1. Serologic responses in mice infected with *B. abortus*

Strain used for infection[a]	Strain used in dot ELISA[b]	Log_{10} antibody titer at (wk postinfection)[c]:			
		4	6	10	20
19	2308	2.20 ± 0.32**	2.74 ± 0.11**	2.68 ± 0.21**	3.04 ± 0.15**
RB51	2308	0*	0.84 ± 0.35*	0.40 ± 0.24*	0*
2308	2308	1.16 ± 0.30**	2.39 ± 0.40**	2.93 ± 0.12**	3.11 ± 0.10**
19	19	2.69 ± 0.21**	3.59 ± 0.20**	2.75 ± 0.12**	3.05 ± 0.05**
RB51	19	0.32 ± 0.16*	1.30 ± 0.17*	1.10 ± 0.32*	0*
2308	19	1.58 ± 0.41**	2.81 ± 0.49**	3.11 ± 0.29*	2.74 ± 0.06**
19	RB51	2.02 ± 0.20**	2.87 ± 0.18**	2.80 ± 0.27**	2.14 ± 0.11
RB51	RB51	0.70 ± 0.43*	0.98 ± 0.30*	1.78 ± 0.08*	1.42 ± 0.27
2308	RB51	1.52 ± 0.39	2.26 ± 0.34**	3.05 ± 0.13**	2.14 ± 0.06

[a] Mice were given strain 19 (10^7 CFU), RB51 (10^7 CFU), or 2308 (10^5 CFU) by i.p. injection.
[b] Serum from mice at 4, 6, 10, or 20 weeks after infection was measured for antibody to γ-irradiated 2308, 19, or RB51 bacteria.
[c] Results are expressed as mean log_{10} titer ± SEM ($n = 5$). Statistical differences in antibody titers to each bacteria were determined among the three groups of infected mice at each time interval. $P \leq 0.01$ for ** versus * by least-squares means analysis.

Serologic testing. Blood was allowed to clot for 6 h at 25°C before centrifugation. Serum samples from each group of 10 mice were divided into five separate pairs of samples, and each pair was pooled to form five samples per group. The serum samples were stored at −70°C. Thawed samples were measured for antibody to γ-irradiated *B. abortus* 2308, 19, or RB51 by a dot enzyme-linked immunosorbent assay (ELISA) with a goat anti-mouse immunoglobulin G (H- and L-chain specific) horseradish peroxidase conjugate (Jackson ImmunoResearch Laboratories, West Grove, Pa.) as described previously (5). Results from the dot ELISA were expressed as mean log_{10} titer and standard error of the mean (SEM).

Measurement of *B. abortus* CFU in spleens and spleen cell proliferative response to *B. abortus*. Spleens were placed on a sterile 60-mesh stainless steel screen and minced with scissors. The cells were washed through the screen into sterile siliconized centrifuge tubes by being rinsed with PBS. Cells from each group of 10 mice were divided into five separate pairs of samples, and each pair was pooled to form five samples per group. The cells were centrifuged for 10 min at 1,400 × *g*, and the small number of contaminating erythrocytes was removed by resuspending the cell pellet in 10 ml of an erythrocyte-lysing buffer (0.15 M NH_4Cl, 0.01 M $KHCO_3$, 0.12 mM EDTA) and incubating the mixture for 5 min at room temperature. The cells were then washed three times in PBS by centrifugation. A 0.2-ml aliquot containing 10^7 cells was removed and added to 3.8 ml of PBS. The remaining cells were placed in RPMI 1640 medium (Gibco, Grand Island, N.Y.), containing L-glutamine, 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 10% fetal bovine serum (Hyclone Laboratories, Inc., Logan Utah), 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 5×10^{-5} M 2-mercaptoethanol. The spleen cells in PBS were lysed by sonication, and the number of CFU in the lysate was determine by plating dilutions on to tryptose agar plates as described previously (16). Results of the colony counts were expressed as mean number of CFU ± SEM per 10^6 spleen cells. A 50-μl aliquot of the spleen cells (10^7 cells per ml) in RPMI 1640 medium was added to each of two separate flat-bottom wells of a 96-well microtiter plate that contained 100 μl of various concentrations of γ-irradiated *B. abortus* 2308, 19, or RB51 (10^8, 10^7, 10^6 bacteria per well). All spleen cell cultures were incubated for 5 days at 37°C under 5% CO_2. Microtiter plates were placed on a Micro Shaker II (Dynatech Laboratories Inc., Alexandria, Va.) every 2 days during the incubations and mixed at an instrument setting of 3.5 for 1 min. After the 5-day incubation, cell cultures were pulsed for 18 h with 1.0 μCi of [^{3}H]thymidine per well. Cells were harvested onto glass filter mats and counted for radioactivity in a liquid scintillation counter. Cell proliferation results were expressed as mean counts per minute ± SEM incorporated by duplicate cultures.

Statistical analysis. Statistical differences in antibody production and spleen cell proliferation to the three strains of *B. abortus* were determined by least-squares means analysis.

Histopathologic testing and immunoperoxidase staining. Spleen tissues were fixed in 10% neutral buffered formalin and processed by routine paraffin embedment before being stained with hematoxylin and eosin for light-microscopic examination. Formalin-fixed unstained sections were incubated for 1 h with antiserum obtained from rabbits that had been hyperimmunized with γ-irradiated 2308, 19, or RB51 bacteria. Rabbit antisera to strains 2308 and 19 was used at a 1:10,000 dilution, and antiserum to strain RB51 was used at a 1:250 dilution in Tris-saline buffer (0.005 M Tris, 0.15 M NaCl [pH 7.6]). After incubation with the antiserum, the tissue sections were processed for immunoperoxidase staining with an avidin-biotin immunostaining kit (Vectastain ABC Kit; Vector Laboratories, Burlingame, Calif.) as described previously (12). Negative controls included spleen tissue sections incubated with no antiserum and sections incubated with a 1:10,000 or 1:250 dilution of normal rabbit serum before sections were processed for immunoperoxidase staining.

RESULTS

Serologic responses. Sera obtained from mice at 2 weeks after infection with strain 19, RB51, or 2308 contained no antibody to strain 19, RB51, or 2308 (data not shown). During 4 to 20 weeks after infection, antibody titers to strain 19 or 2308 in RB51-infected mice were significantly ($P \leq 0.01$) lower than the titers in mice infected with either strain 19 or strain 2308 (Table 1). In addition, during 4 to 10 weeks after infection, antibody titers to strain RB51 in RB51-infected mice were significantly ($P \leq 0.01$) lower than the titers in mice infected with either strain 19 or strain 2308. Mice infected with strain 19 or 2308 had measurable antibody titers to either 19, 2308, or RB51 at 20 weeks after infection, whereas RB51-infected mice had a titer to only RB51 at 20 weeks (Table 1).

Numbers of *B. abortus* CFU in spleen. Mice infected with strain RB51 exhibited a rapid decline in numbers of bacteria in

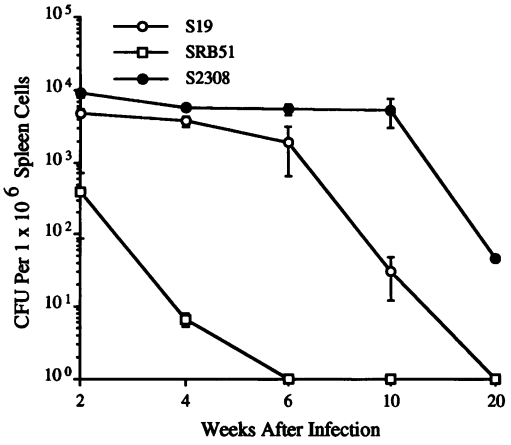

FIG. 1. Persistence of *B. abortus* in spleens of infected mice. Mice were given strain 19 (S19) (10^7 CFU), RB51 (SRB51) (10^7 CFU), or 2308 (S2308) (10^5 CFU) by i.p. injection. Spleen cell suspensions were lysed by sonication and plated onto trypose agar plates to determine numbers of CFU at 2 to 20 weeks after infection. Results are presented as mean CFU ± SEM (n = 5).

their spleens between 2 and 4 weeks after infection, and at 6 weeks after infection no bacteria were cultured from the spleens (Fig. 1). Mice infected with strain 19 had relatively constant numbers of bacteria in their spleens between 2 and 6 weeks after infection. Numbers of bacteria then declined sharply at 10 weeks, and no bacteria were cultured from the spleens at 20 weeks after infection. In contrast to strains RB51 and 19, mice infected with 2308 exhibited little change in numbers of bacteria in the spleens between 2 and 10 weeks and bacteria were still present in the spleens at 20 weeks after infection (Fig. 1).

Spleen cell proliferative response to strain 2308. Spleen cells obtained from noninfected control mice and mice at 2 weeks after infection with either 2308, 19, or RB51 did not have significantly different proliferative responses when incubated with 2308 bacteria (data not shown). At 4 weeks after infection, however, spleen cells from RB51-infected mice, but not from mice infected with strain 19 or 2308, exhibited significantly ($P \leq 0.01$) increased proliferative responses to 2308 when compared with control mice (Fig. 2A). At 6 weeks after infection, spleen cells obtained from mice infected with strain RB51 or 19 but not from mice infected with strain 2308 exhibited significantly ($P \leq 0.01$) increased proliferative responses to strain 2308 (Fig. 2B). Spleen cells from mice at 6 weeks after infection with strain 19 or RB51 exhibited significant ($P \leq 0.01$) and similar proliferative responses when incubated with the highest concentrations of strain 2308 (10^8 or 10^7 bacteria). However, only cells from mice infected with strain 19 had a significantly ($P \leq 0.01$) increased response when incubated with the lowest concentration of strain 2308 (10^6 bacteria [Fig. 2B]). Spleen cells from strain 2308-infected mice did not have significantly ($P \leq 0.01$) increased proliferative responses to strain 2308 until 10 weeks after infection when compared with control mice (Fig. 2C). Cells from mice infected with strain 19 or RB51 also had significantly increased ($P \leq 0.01$) proliferative responses to strain 2308 at 10 weeks after infection. However, the proliferative response of spleen cells from mice following 10 weeks of infection with the three strains of *B. abortus* was related to the stimulating concentration of strain 2308 bacteria. Spleen cells from strain 2308-infected mice proliferated when incubated with 10^8, 10^7, or 10^6 strain 2308 bacteria; cells from strain 19-infected mice proliferated only in response to 10^8 or 10^7 bacteria, and cells from strain RB51-infected mice proliferated when incubated only with 10^8 bacteria (Fig. 2C). At 20 weeks after infection, cells from mice infected with strain 2308 or 19 but not from mice infected with strain RB51 exhibited significantly increased ($P \leq 0.01$) proliferative responses when incubated with all tested concentrations of strain 2308 (Fig. 2D).

Spleen cell proliferative response to strain 19. Throughout the 20-week study, spleen cells taken from mice infected with strain 19, 2308, or RB51 exhibited proliferative responses, when incubated with strain 19 bacteria (data not shown), which were similar to those that occurred when cells were incubated with strain 2308 bacteria (Fig. 2).

Spleen cell proliferative response to strain RB51. Spleen cells obtained from noninfected control mice and mice at 2 weeks after infection with strain 2308, 19, or RB51 did not have significantly different proliferative responses when incubated with strain RB51 bacteria (data not shown). However, spleen cells from mice at 4 weeks after infection with strain 2308, 19, or RB51 exhibited significantly ($P \leq 0.01$) increased proliferative responses following incubation with RB51 compared with the responses of spleen cells from control mice (Fig. 3A). At 4 weeks, spleen cells from strain 2308-infected mice proliferated in response to only the highest tested RB51 concentration (10^8 bacteria), whereas cells from mice infected with strain 19 or RB51 proliferated in response to all concentrations (10^8, 10^7, or 10^6 bacteria) of RB51 (Fig. 3A). Significantly ($P \leq 0.01$) increased proliferative responses also occurred when RB51 was incubated with spleen cells taken from mice at 6 weeks (Fig. 3B) and 10 weeks (Fig. 3C) after infection with strain 19, RB51, or 2308. During this time, the proliferative response to strain RB51 by spleen cells from strain RB51-infected mice was usually similar to the responses of spleen cells obtained from mice infected with strain 19 or 2308. However, at 20 weeks after infection, spleen cells from mice infected with strain 19 or 2308, but generally not cells from mice infected with strain RB51, exhibited significantly ($P \leq 0.01$) increased proliferative responses when incubated with RB51 bacteria (Fig. 3D).

Histopathologic testing and immunoperoxidase staining. Spleens of mice at 2 weeks after infection with strain 2308 or 19 had profound lymphoid depletion of the white pulp, small germinal centers with few mitotic figures, and multifocal accumulations of large macrophages that surrounded and sometimes covered the periarteriolar lymphoid sheaths. In contrast, spleens of mice at 2 weeks after infection with strain RB51 had little to no lymphoid depletion, fewer macrophages, and the appearance of germinal centers with numerous mitotic figures (results not shown). At 4 weeks, the germinal centers in the spleens of strain RB51-infected mice occasionally occupied up to two-thirds of the total area of the associated periarteriolar lymphoid sheath and the germinal centers were much larger than those seen in mice infected with strain 19 or 2308 (results not shown). Large germinal centers and the disappearance of the macrophage accumulations in the spleens did not occur until 6 to 10 weeks after infection of mice with strain 19 and until 10 weeks after infection of mice with strain 2308. Immunoperoxidase staining revealed that strain RB51 antigens persisted in the spleens for 4 weeks after infection of mice with strain RB51. Mice infected with strain 19 contained strain 19 antigens in the spleens for up to 6 weeks after infection. In contrast, strain 2308 antigens persisted in the spleens of strain 2308-infected mice throughout the 20-week study. For all three strains, the bacterial antigens in the spleens were confined to

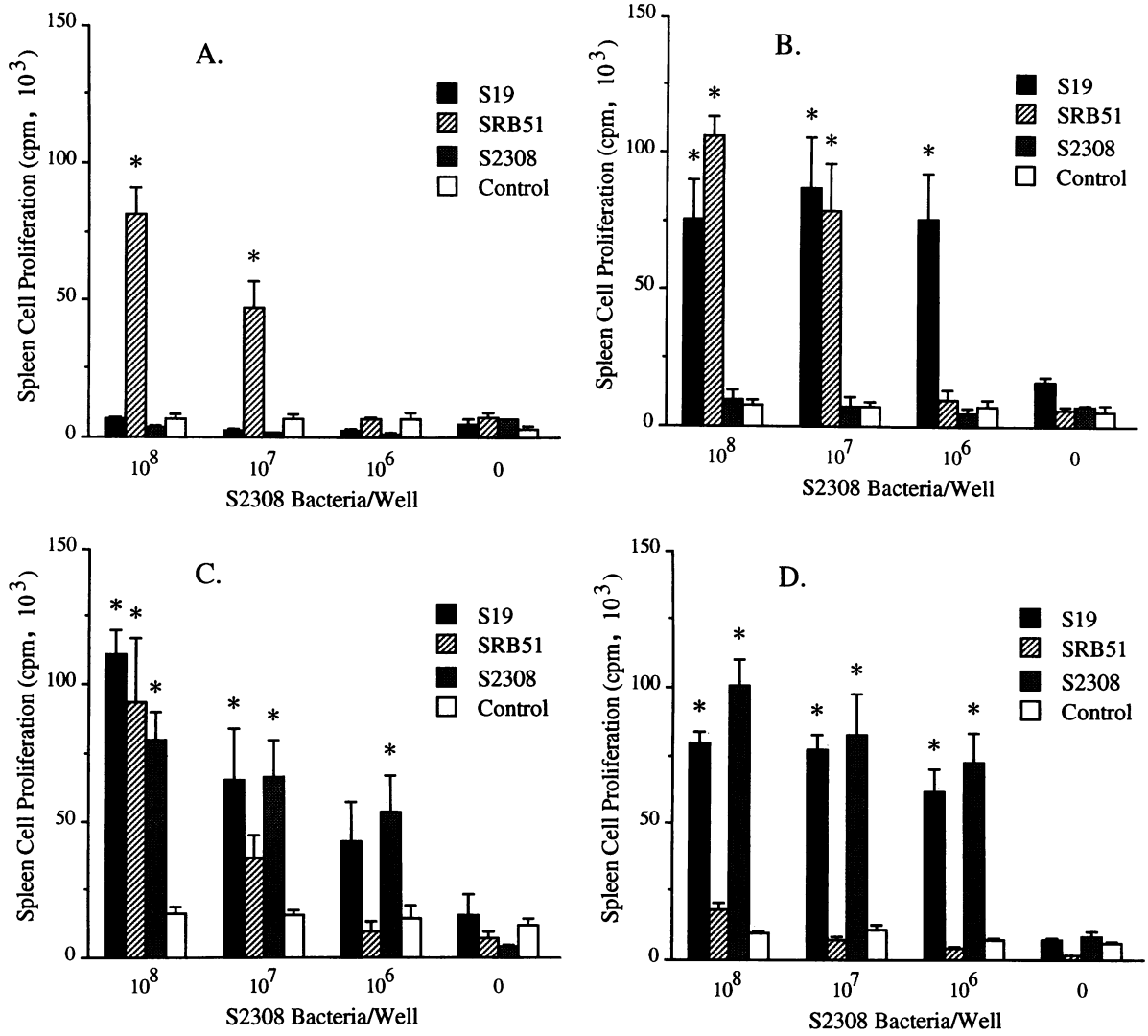

FIG. 2. Spleen cell proliferation in response to *B. abortus* 2308 in mice infected with *B. abortus* 19, RB51, or 2308. Mice were given strain 19 (S19) (10^7 CFU), RB51 (SRB51) (10^7 CFU), or 2308 (S2308) (10^5 CFU) by i.p. injection. Spleen cell suspensions were prepared from mice at 4 (A), 6 (B), 10 (C), or 20 (D) weeks after infection. Cells (3×10^5 cells per well) were incubated for 5 days with 10^8 to 10^6 γ-irradiated killed strain 2308 bacteria and then pulsed for 18 h with [^{3}H]thymidine. Results are presented as mean counts per minute (cpm) $\pm$ SEM (n = 5). $P \leq 0.01$ for * versus controls for each strain 2308 concentration by least-squares means analysis.

the cytoplasm of the macrophage that surrounded the periarteriolar lymphoid sheaths (not shown).

DISCUSSION

Mice infected with strain RB51 produced antibodies that reacted with the surface antigens of strain 2308. However, throughout the 20-week study strain RB51-infected mice had lower antibody titers to strain 2308 than did mice infected with strain 19 or 2308. The antibody responses in RB51-infected mice were probably directed primarily to the outer membrane proteins of 2308, because previous studies have shown that RB51-infected mice produce antibodies to the outer membrane proteins (21) but not to the LPS O antigens of strain 2308 (21, 26). Mice infected with strain RB51, 19, or 2308 generally exhibited similar spleen cell proliferative responses to strain 2308 at 6 and 10 weeks after infection. However, differences occurred during the 20-week study in that the responses in RB51-infected mice appeared sooner (present at 4 weeks) and were less persistent (absent at 20 weeks) than the responses in mice infected with strain 19 or 2308.

The lower antibody titers and shorter persistence of cell-mediated immune responses in spleens from RB51-infected mice probably resulted because live RB51 bacteria and antigens of RB51 were cleared much more rapidly from the spleen than for either strain 19 or strain 2308. Culture analysis and immunoperoxidase staining of spleen tissues revealed that RB51 bacteria and bacterial antigens disappeared from the spleen at 6 weeks and that strain 19 and antigens of strain 19 were absent from the spleen at 20 weeks after infection. In contrast, at 20 weeks, live 2308 bacteria were still present in the spleen. Antigens of strains 19 and RB51 did not persist after the bacteria had been killed in the spleen. Therefore, persistence of live bacteria, but not antigens from killed bacteria, probably maintained stimulation of the immune response in the infected mice. Other studies, which have used similar

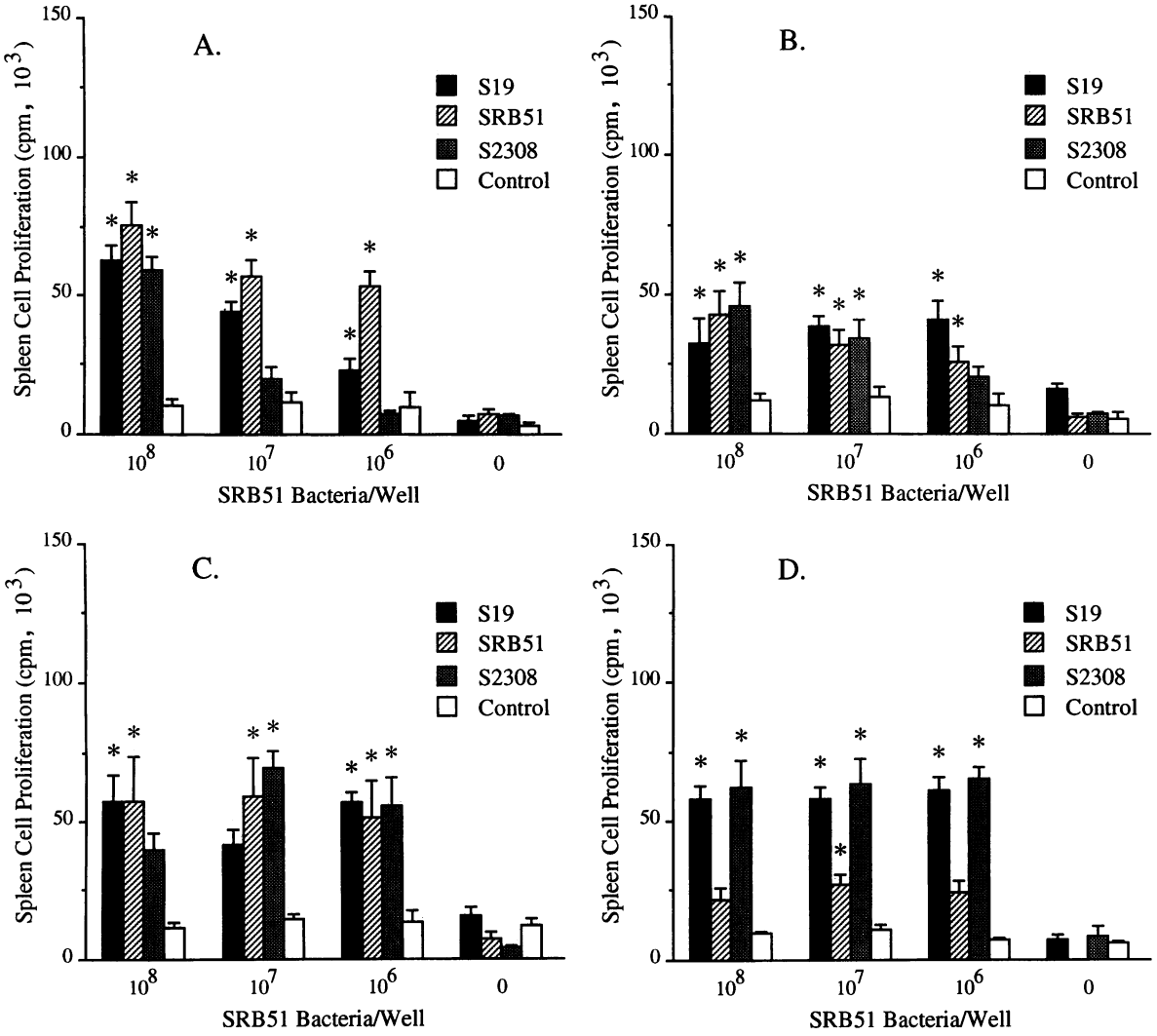

FIG. 3. Spleen cell proliferation in response to *B. abortus* RB51 in mice infected with *B. abortus* 19, RB51, or 2308. Mice were given strain 19 (S19) (10^7 CFU), RB51 (SRB51) (10^7 CFU), or 2308 (S2308) (10^5 CFU) by i.p. injection. Spleen cell suspensions were prepared from mice at 4 (A), 6 (B), 10 (C), or 20 (D) weeks after infection. Cells (3×10^5 cells per well) were incubated for 5 days with 10^8 to 10^6 γ-irradiated killed strain RB51 bacteria and then pulsed for 18 h with [^{3}H]thymidine. Results are presented as mean counts per minute (cpm) ± SEM (n = 5). $P \leq 0.01$ for * versus controls for each strain RB51 concentration by least-squares means analysis.

infective doses of the three strains of *B. abortus*, have shown that the persistence of bacteria in the spleens of infected mice is at least 24 weeks for strain 2308 (7, 13), approximately 8 to 12 weeks for strain 19 (1, 13, 18), and 4 weeks for strain RB51 (21).

The RB51 mutant was considerably less virulent than its parental strain, 2308, because RB51 was cleared from the spleen at 4 weeks whereas 2308 persisted in the spleen for at least 20 weeks after infection. This large difference in the clearance rate occurred even though mice were infected with approximately 100 times more RB51 than 2308. The mechanism responsible for the reduced virulence of RB51 and its rapid clearance from infected mice is not known. Rough strains of *B. abortus* that resemble RB51 are more rapidly ingested (2) and killed by macrophages than are strains 19 and 2308 (2, 8, 15). In addition, both strains 19 and 2308 appear to induce suppressor macrophages in the spleens (3, 10, 18), whereas rough strains of *B. abortus* do not (3). Therefore, rapid clearance of strain RB51 in the current study may have resulted from this bacterium being more easily killed by macrophages and having less immunosuppressive activity than strains 19 and 2308.

At 4 weeks after infection, RB51-infected mice exhibited spleen cell proliferative responses to 2308, although no responses occurred in mice infected with strain 19 or 2308. This probably resulted because at 4 weeks spleens of strain RB51-infected mice had large germinal centers and no lymphoid depletion whereas spleens of mice infected with strain 19 or 2308 had small or no germinal centers and moderate to severe lymphoid depletion. Previous studies have shown that severe lymphoid depletion occurs in the spleen at 3 to 6 weeks after infection of mice with strain 19 or 2308 (7, 17), and during the depletion spleen cells are incapable of exhibiting cell-mediated immune responses to 2308 (1, 10). Therefore, in the current study, spleen cell proliferation in response to strain 2308 at 4 weeks in RB51-infected mice but not in mice infected with strain 19 or 2308 probably occurred because RB51 was the only infecting strain that did not induce lymphoid depletion in the

spleen. In strain 19-infected mice, spleen cell proliferation in response to strain 2308 at 6 weeks was associated with the disappearance of lymphoid deletion in the spleen. Similarly, the disappearance of lymphoid depletion at 10 weeks in spleens of strain 2308-infected mice coincided with the capacity of the cells to proliferate in response to 2308. Thus, lymphoid depletion after infection with strain 19 or 2308 was associated with reduced spleen cell proliferation in response to 2308.

Mice at 4 to 6 weeks after infection with strain 19 or 2308 exhibited lymphoid depletion in the spleens, and the spleen cells proliferated when incubated with strain RB51 but not with strain 19 or 2308. The reason for this finding is not known, although the absence of the LPS O side chain in strain RB51 is the most likely explanation. The LPS O side chain of *B. abortus* acts as a virulence factor because rough strains are less virulent (8, 15) and less effective in stimulating suppressor macrophages and are more easily ingested and killed by macrophages than are smooth strains of *B. abortus* (2, 3, 8, 15). In the present study, spleen cells from mice infected with strain 19 or 2308 might have proliferated in response to RB51 but not to 19 or 2308 because strain RB51 was more easily ingested and processed by macrophages and other antigen-presenting cells than were strains 19 and 2308. In addition, strain RB51 might have been less effective than strains 19 and 2308 in stimulating suppressor macrophages. The LPS O side chain of some gram-negative bacteria is also thought to be immunosuppressive by stimulating suppressor T cells (9). However, it is not known if the LPS O side chain of *B. abortus* exhibits similar activity and if strain RB51 is less effective in stimulating suppressor T-cell activity than are strains 19 and 2308.

In the present study, we measured antibody and cell-mediated immune responses to strains RB51, 19, and 2308 in mice infected with each of these three strains to determine if major differences occurred in the cross-reactivity of the immune response among the strains. Mice infected with any one of the three *B. abortus* strains produced antibody and proliferating spleen cells that reacted with the other two strains at some point during the 20-week study. These types of results would be expected on the basis of the similarity of the proteins in strains 19, RB51, and 2308. These three strains have the same outer membrane proteins (19, 21) and exhibit an 86 to 91% similarity in their total cellular proteins as shown by two-dimensional gel electrophoresis (22).

Mice given strain RB51 have enhanced resistance to infection when challenged 7 weeks later with strain 2308, even though RB51 is cleared from the spleen by 4 weeks after infection (21). Similar results have been reported for cattle given strain RB51, in that RB51 is cleared from the lymph nodes within 4 to 6 weeks (4, 5) but the cattle have lymph node proliferative responses in vitro to strain 2308 at 10 and 12 weeks (24) and have enhanced resistance to abortions following challenge with 2308 (5). The capacity of strain RB51 to enhance resistance and induce immune responses to strain 2308 despite its rapid clearance from animals is an important issue that will have to be addressed in evaluating the possible replacement of strain 19 with strain RB51 as a vaccine for preventing brucellosis in cattle. As reported here, mice given RB51 had less persistent antibody and cell-mediated immune responses to strain 2308 than did mice given strain 19. However, these results cannot be extrapolated to mean that strain RB51 would be less effective than strain 19 in protecting either mice or cattle from infection with 2308. Animals given RB51 may be adequately protected from infection with 2308 by mounting an anamnestic response after dissipation of the vaccine-induced immune response. Studies evaluating these types of responses and the duration of protection from strain 2308 infection in mice and cattle given RB51 are in progress.

REFERENCES

1. **Araya, L. N., P. H. Elzer, G. E. Rowe, F. M. Enright, and A. J. Winter.** 1989. Temporal development of protective cell-mediated and humoral immunity in BALB/c mice infected with *Brucella abortus*. J. Immunol. **143:**3330–3337.
2. **Braun, A. I., A. Pomales-Lebron, and W. R. Stinebring.** 1958. Interaction between mononuclear phagocytes and *Brucella abortus* strains of different virulence. Proc. Soc. Exp. Biol. Med. **97:**393–397.
3. **Cheers, C., H. Pavlov, C. Riglar, and E. Madraso.** 1980. Macrophage activation during experimental murine brucellosis. III. Do macrophages exert feedback control during brucellosis? Cell. Immunol. **49:**168–177.
4. **Cheville, N. F., A. E. Jensen, S. M. Halling, F. M. Tatum, D. C. Morfitt, S. G. Hennager, W. M. Frerichs, and G. Schurig.** 1992. Bacterial survival, lymph node changes, and immunologic responses of cattle vaccinated with standard and mutant strains of *Brucella abortus*. Am. J. Vet. Res. **53:**1881–1888.
5. **Cheville, N. F., M. G. Stevens, A. E. Jensen, F. M. Tatum, and S. M. Halling.** 1993. Immune responses and protection against infection and abortion in cattle experimentally vaccinated with mutant strains of *Brucella abortus*. Am. J. Vet. Res. **54:**1591–1597.
6. **Enright, F. M.** 1990. The pathogenesis and pathobiology of *Brucella* infection in domestic animals, p. 301–320. *In* K. Nielsen and J. R. Duncan (ed.), Animal brucellosis. CRC Press, Inc., Boca Raton, Fla.
7. **Enright, F. M., L. N. Araya, P. H. Elzer, G. E. Rowe, and A. J. Winter.** 1990. Comparative histopathology in BALB/c mice infected with virulent and attenuated strains of *Brucella abortus*. Vet. Immunol. Immunopathol. **26:**171–182.
8. **Harmon, B. G., L. G. Adams, and M. Frey.** 1988. Survival of rough and smooth strains of *Brucella abortus* in bovine mammary gland macrophages. Am. J. Vet. Res. **49:**1092–1097.
9. **Haslov, K., A. Fomsgaard, K. Takayama, J. S. Fomsgaard, P. Ibsen, M. B. Fauntleroy, P. W. Stashak, C. E. Taylor, and P. J. Baker.** 1992. Immunosuppressive effects induced by the polysaccharide moiety of some bacterial lipopolysaccharides. Immunobiology **186:**378–393.
10. **Lauderdale, T. L., S. M. Jones, and A. J. Winter.** 1990. Response of murine T cells to antigens of *Brucella abortus* at sequential periods after infection. Immunol. Infect. Dis. **1:**59–66.
11. **MacMillan, A.** 1990. Conventional serological tests, p. 153–197. *In* K. Nielsen and J. R. Duncan (ed.), Animal brucellosis. CRC Press, Inc., Boca Raton, Fla.
12. **Meador, V. P., L. B. Tabatabai, W. A. Hagemoser, and B. L. Deyoe.** 1986. Identification of *Brucella abortus* in formalin-fixed, paraffin-embedded tissues of cows, goats, and mice with an avidin-biotin-peroxidase complex immunoenzymatic staining technique. Am. J. Vet. Res. **47:**2147–2150.
13. **Montaraz, J. A., and A. J. Winter.** 1986. Comparison of living and nonliving vaccines for *Brucella abortus* in BALB/c mice. Infect. Immun. **53:**245–251.
14. **Nicoletti, P.** 1990. Vaccination, p. 283–300. *In* K. Nielsen and J. R. Duncan (ed.), Animal brucellosis. CRC Press, Inc., Boca Raton, Fla.
15. **Price, R. E., J. W. Templeton, and L. G. Adams.** 1990. Survival of smooth, rough and transposon mutant strains of *Brucella abortus* in bovine mammary macrophages. Vet. Immunol. Immunopathol. **26:**353–365.
16. **Pugh, G. W., Jr., L. B. Tabatabai, M. Phillips, and T. J. McDonald.** 1991. Establishment of dose-response relationships in BALB/c mice using *Brucella* cell surface protein and lipopolysaccharide. Am. J. Vet. Res. **52:**261–268.
17. **Pugh, G. W., Jr., E. S. Zehr, V. P. Meador, M. Phillips, T. J. McDonald, and B. L. Deyoe.** 1989. Immunologic, histopathologic, and bacteriologic responses of five strains of mice to *Brucella abortus* strain 2308. Am. J. Vet. Res. **50:**323–328.
18. **Riglar, C., and C. Cheers.** 1980. Macrophage activation during experimental murine brucellosis. II. Inhibition of in vitro lympho-

cyte proliferation by *Brucella*-activated macrophages. Cell. Immunol. **49:**154–167.
19. **Santos, J. M., D. R. Verstreate, V. Y. Perera, and A. J. Winters.** 1984. Outer membrane proteins from rough strains of four *Brucella* species. Infect. Immun. **46:**188–194.
20. **Schurig, G. G., C. Hammerberg, and B. R. Finkler.** 1984. Monoclonal antibodies to *Brucella* surface antigens associated with the smooth lipopolysaccharide complex. Am. J. Vet. Res. **45:**967–971.
21. **Schurig, G. G., R. M. Roop, T. Bagchi, S. Boyle, D. Buhrman, and N. Sriranganathan.** 1991. Biological properties of RB51: a stable rough strain of *Brucella abortus*. Vet. Microbiol. **28:**171–188.
22. **Sowa, B. A., K. A. Kelly, T. A. Ficht, and G. Adams.** 1992. Virulence associated proteins of *Brucella abortus* identified by paired two-dimensional gel electrophoretic comparisons of virulent, vaccine, and LPS deficient strains. Appl. Theor. Electrophor. **3:**33–40.
23. **Stevens, M. G., S. G. Hennager, S. C. Olsen, and N. F. Cheville.** 1994. Serologic responses in diagnostic tests for brucellosis in cattle vaccinated with *Brucella abortus* 19 or RB51. J. Clin. Microbiol. **32:**1065–1066.
24. **Stevens, M. G., S. C. Olsen, and N. F. Cheville.** Comparative analysis of immune responses in cattle vaccinated with *Brucella abortus* strain 19 or RB51. Vet. Immunol. Immunopathol., in press.
25. **Sutherland, S. S., and J. Searson.** 1990. The immune response to *Brucella abortus*: the humoral immune response, p. 65–81. *In* K. Nielsen and J. R. Duncan (ed.), Animal brucellosis. CRC Press, Inc., Boca Raton, Fla.
26. **Tobias, L., G. G. Schurig, and D. O. Cordes.** 1992. Comparative behaviour of *Brucella abortus* strains 19 and RB51 in the pregnant mouse. Res. Vet. Sci. **53:**179–183.
27. **Young, E. J.** 1983. Human brucellosis. Rev. Infect. Dis. **5:**821–842.