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Comparison of Immune Responses and Resistance to Brucellosis in Mice Vaccinated with *Brucella abortus* 19 or RB51

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Immune responses and resistance to infection with *Brucella abortus* 2308 (S2308) were measured in mice following vaccination with *B. abortus* 19 (S19) or the lipopolysaccharide (LPS) O-antigen-deficient mutant, strain RB51 (SRB51). Live bacteria persisted for 8 weeks in spleens of mice vaccinated with 5×10^6 or 5×10^8 CFU of SRB51, whereas bacteria persisted for 12 weeks in mice vaccinated with 5×10^6 CFU of S19. Mice vaccinated with 5×10^6 or 5×10^8 CFU of SRB51 had increased resistance to infection with S2308 at 12, 16, and 20 weeks after vaccination, but the resistance was lower than that induced by vaccinating mice with 5×10^6 CFU of S19. Spleen cells obtained from mice vaccinated with S19 or SRB51 generally exhibited similar proliferative responses to S2308 bacteria or bacterial proteins (106 to 18 kDa) following challenge of mice with S2308 at 12, 16, or 20 weeks after vaccination. Mice vaccinated with S19 had antibody to S2308 bacteria and S2308 smooth LPS at 4, 8, and 12 weeks after vaccination. In contrast, mice vaccinated with either dose of SRB51 did not produce antibody to S2308 smooth LPS. In addition, only mice vaccinated with the highest dose of SRB51 (5×10^8 CFU) had antibody responses to S2308 bacteria, although the responses were lower and less persistent than those in mice vaccinated with S19. Collectively, these results indicate that SRB51-vaccinated mice have similar cell-mediated immune responses to S2308 but lower resistance to infection with S2308 compared with S19-vaccinated mice. The lower resistance in SRB51-vaccinated mice probably resulted from a combination of rapid clearance of SRB51 and an absence of antibodies to S2308 LPS.

Cattle are vaccinated with *Brucella abortus* 19 (S19) to prevent abortions that are caused by infections with field strains of *B. abortus* (9). The S19 vaccine has an important disadvantage, however, because it induces production of antibodies to the lipopolysaccharide (LPS) O antigens of *B. abortus* that can be detected by serodiagnostic tests for brucellosis in cattle (6, 23). Consequently, it can be difficult to distinguish between S19-vaccinated and naturally infected cattle by using serologic tests when attempting to identify and remove cattle with brucellosis from vaccinated herds (6).

B. abortus RB51 (SRB51) is a laboratory-derived rough mutant of the virulent strain 2308 (S2308) of *B. abortus* (15). SRB51 contains the same outer membrane proteins as do S19 and S2308 (13, 15), and it also appears to contain all S2308 proteins that stimulate cell-mediated immune responses in cattle (17). However, SRB51 differs from S19 and S2308 in that it does not contain the LPS O antigens (12, 14, 15). Therefore, cattle vaccinated with SRB51 do not produce antibodies to the O antigens of *B. abortus* that are detected by serologic tests for brucellosis (3, 4, 15, 16). Cattle vaccinated with SRB51 also have cell-mediated immune responses to S2308 bacteria (18) and appear to be protected from abortions following challenge with S2308 in a similar manner as occurs when cattle are vaccinated with S19 (4). These attributes suggest that SRB51 might be better than S19 as a vaccine in cattle, because SRB51 induces immunity without inducing serologic responses to LPS that complicate the interpretation of diagnostic tests for brucellosis.

Mice vaccinated with SRB51 have enhanced resistance to infection with S2308 (15). However, a comparative analysis of the protection from infection with S2308 in mice vaccinated with SRB51 or S19 has not been undertaken. We previously reported that SRB51 is considerably less pathogenic and is cleared more rapidly from mice than is S19 and that SRB51 induces lower and less persistent immune responses to S2308 and S2308 proteins in mice than does S19 (19, 20). On the basis of these results, we previously hypothesized that mice vaccinated with SRB51 or S19 may have similar extents of resistance to brucellosis, provided that SRB51 exhibits one of the following properties. First, the immunogenicity of SRB51 can be increased to that of S19 by increasing the vaccine dose of SRB51. Second, even though SRB51 may be less immunogenic than S19, it may be sufficiently immunogenic such that a protective anamnestic response occurs after infection with S2308. These two hypotheses were tested in the current study by vaccinating mice either with an equal dose of S19 or SRB51 or with a 100-fold-higher dose of SRB51 and measuring resistance to infection and immune responses following challenge with S2308.

MATERIALS AND METHODS

Culture medium. All experiments used RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing L-glutamine, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 10% fetal bovine serum (HyClone Laboratories, Logan, Utah), 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 5×10^{-5} M 2-mercaptoethanol. This supplemented medium will subsequently be referred to as RPMI.

***B. abortus* cultures and proteins.** Live cultures of S2308, S19, and SRB51 (18) and killed (γ irradiation, 1.4×10^6 rads) cultures of S2308 (20) were prepared as described previously. Whole-cell lysates were prepared from killed S2308 and separated into 106- to 18-kDa proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (18). The S2308 proteins were eluted from the gel into 22 fractions by using a Blotelutor B35 (Biometra, Gottingen, Germany). Each protein fraction was concentrated and washed by membrane filtration as described previously (18) and then filter sterilized and added to RPMI medium. The fractions contained approximately 5 to 25 μg of the S2308 proteins per ml. A 50-μl aliquot of each of the 22 protein fractions of S2308 was

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added to two separate wells of a 96-well flat-bottom microtiter plate, and plates were stored at −70°C.

Vaccination and challenge of mice with *B. abortus*. Female 8-week-old BALB/c AnNHsD mice were obtained from Harlan Sprague Dawley (Indianapolis, Ind.) and were used in the experiments when 12 weeks old. Mice (eight per treatment group) were injected intraperitoneally with 0.2 ml of a 0.15 M NaCl saline solution (controls) or 0.2 ml of saline containing approximately 5×10^6 CFU of S19 or 5×10^6 or 5×10^8 CFU of SRB51. Nonvaccinated control mice and vaccinated mice at 12, 16, or 20 weeks after vaccination were challenged with S2308 by an intraperitoneal injection of approximately 2×10^4 to 4×10^4 CFU in 0.2 ml of NaCl saline. The precise numbers of CFU used for vaccination or challenge were determined retrospectively by viable plate counts and found to be 4.2×10^6 for S19, 5.7×10^6 and 4.8×10^8 for SRB51, and 2.4×10^4 to 4.5×10^4 for S2308.

Collection of tissues. Blood samples and spleens were obtained from age-matched nonvaccinated control mice and from vaccinated-mice at 2, 4, 8, or 12 weeks after vaccination. In addition, blood and spleens were obtained from nonvaccinated control mice and from vaccinated mice at 2 weeks after they were challenged with S2308. Blood was allowed to clot for 6 h at 25°C before centrifugation. Serum samples were then stored at −70°C. Spleens were weighed, approximately one-third of the spleen was excised, and the excised portion was weighed. The excised portion of the spleen was used for bacterial culture, and the remaining portion was used to prepare spleen cell suspensions.

Serologic testing. Thawed serum samples were measured for antibody to γ-irradiated *B. abortus* S2308 by a dot enzyme-linked immunosorbent assay (ELISA) and for antibody to S2308 smooth LPS by an ELISA as described previously (5, 21). Both procedures used a goat anti-mouse immunoglobulin G (H- and L-chain specific)-horseradish peroxidase conjugate (Jackson ImmunoResearch Laboratories, West Grove, Pa.). Results from the dot ELISA and ELISA were expressed as mean $\log_{10}$ titer ± standard deviation (SD) and mean optical density ± SD, respectively.

Culture analysis. The excised portion of the spleen was processed to form a cell lysate by using a tissue grinder, and the number of *B. abortus* CFU in the lysate was determined by plating dilutions onto tryptose agar plates as described previously (11). The number of CFU per total spleen was determined by the following formula: (total spleen weight/spleen portion weight) × number of CFU in spleen portion. Results were then expressed as mean $\log_{10}$ CFU per total spleen ± standard error of the mean (SEM).

Preparation of spleen cell suspensions. After removal of part of the spleen for culture analysis, the remaining portion was placed on a sterile 60-mesh stainless steel screen, minced with scissors, and processed to form a spleen cell suspension as described previously (20). Cells from each group of eight mice were divided into four separate pairs of samples, and each pair was pooled to form four samples per group. The spleen cells were then placed in RPMI.

Measurement of spleen cell proliferation to S2308 and S2308 proteins. Fifty μl of RPMI containing 3×10^5 spleen cells 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* S2308 (10^7, 10^6, or 10^5 bacteria per well). Plates containing the 22 isolated S2308 protein fractions (106 to 18 kDa) were thawed, and 50 μl of RPMI containing 3×10^5 spleen cells was added to each well. All spleen cell cultures were incubated with S2038 bacteria or S2308 bacterial proteins for 5 days at 37°C in 5% CO_2. Cell cultures were mixed on days 2 and 4 by shaking microtiter plates for 1 min on a Micro Shaker II (Dynatech Laboratories Inc., Alexandria, Va.), using an instrument setting of 3.5. After the 5-day incubation, cell cultures were pulsed for 18 h with 1.0 μCi of [^{3}H]thymidine per well. Cells were then harvested and measured for radioactivity in counts per minute in a liquid scintillation counter. Cell proliferation results were expressed as mean counts per minute ± SEM incorporated by duplicate cultures.

Statistical analysis. Statistical differences among nonvaccinated control mice or mice vaccinated with S19 or SRB51 were determined by analysis of variance and Fisher's protected least significant difference.

RESULTS

Persistence of bacteria in spleen and spleen weights in vaccinated mice. Mice given 5×10^6 or 5×10^8 CFU of SRB51 exhibited similar clearance of bacteria from the spleen, and both groups of mice generally had significantly ($P \leq 0.01$) lower numbers of bacteria in the spleen than did mice given 5×10^6 CFU of S19 (Fig. 1A). The only exception occurred at 8 weeks, when mice given 5×10^8 CFU of SRB51 or 5×10^6 CFU of S19 had similar and significantly ($P \leq 0.01$) higher numbers of bacteria in the spleen than did mice given 5×10^6 CFU of SRB51 (Fig. 1A). No bacteria were cultured from the spleen at 12 weeks in both groups of SRB51-vaccinated mice. In contrast, S19-vaccinated mice had low amounts of bacteria in the spleen at 12 weeks (Fig. 1A), and clearance of bacteria from the spleen did not occur until 16 weeks after vaccination

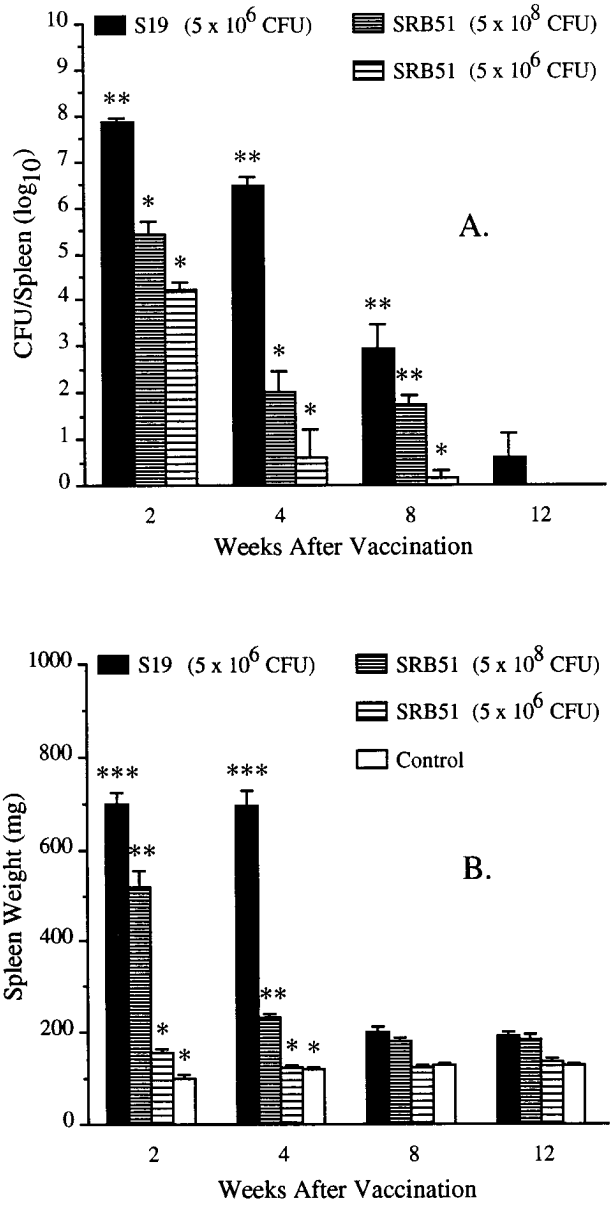

FIG. 1. Persistence of bacteria in spleen and spleen weights in vaccinated mice. Mice were vaccinated with S19 (5×10^6 CFU) or SRB51 (5×10^6 or 5×10^8 CFU). Numbers of bacteria in the spleen (A) and spleen weights (B) were measured at 2, 4, 8, and 12 weeks after vaccination. Results are expressed as mean ± SEM ($n = 8$). Groups with different numbers of asterisks are different ($P \leq 0.01$) by analysis of variance and Fisher's protected least significant difference.

(data not shown). Spleen weights in S19-vaccinated mice at 2 and 4 weeks were significantly ($P \leq 0.01$) higher than spleen weights in either nonvaccinated control mice or mice vaccinated with either dose of SRB51 (Fig. 1B). Among SRB51-vaccinated mice, only those given the largest dose of SRB51 (5×10^8 CFU) had significantly ($P \leq 0.01$) higher spleen weights at 2 and 4 weeks after vaccination compared with control mice. Spleens in all vaccinated mice at 8 and 12 weeks were not different in weight from spleens of nonvaccinated control mice (Fig. 1B).

Resistance to infection and spleen weights in vaccinated mice following challenge with S2308. Mice vaccinated with S19

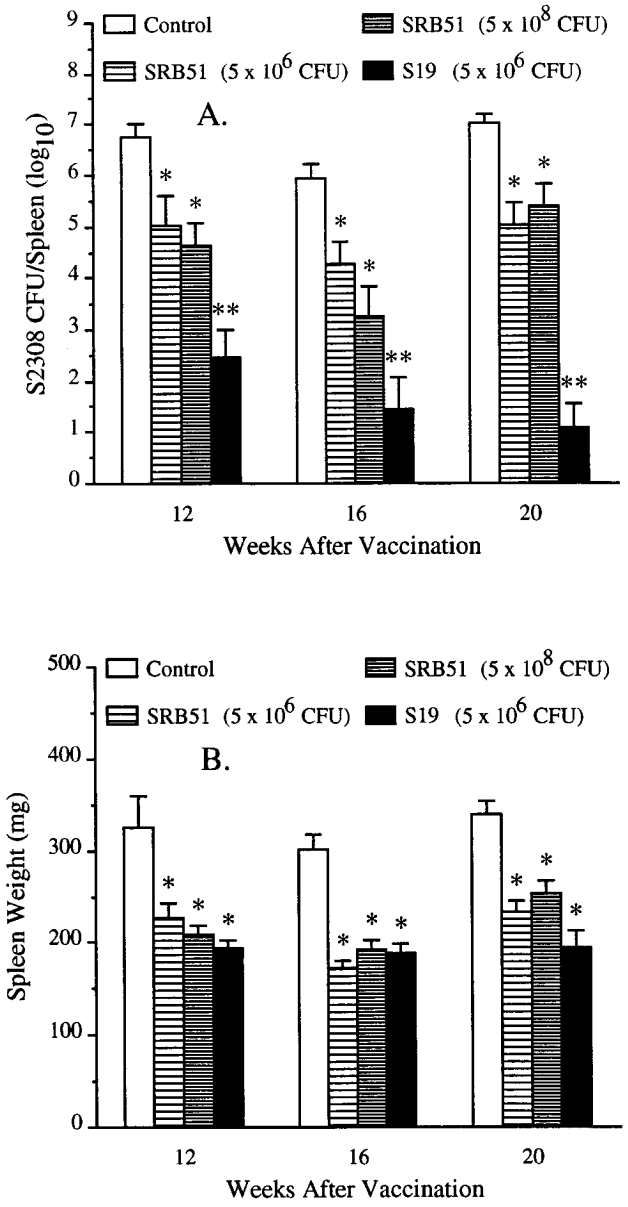

FIG. 2. Resistance to infection and spleen weights in vaccinated mice following challenge with S2308. Mice were challenged with S2308 at 12, 16, or 20 weeks after vaccination with S19 (5×10^6 CFU) or SRB51 (5×10^6 or 5×10^8 CFU). Numbers of S2308 CFU per spleen (A) and spleen weights (B) were measured at 2 weeks after challenge. Results are expressed as mean ± SEM ($n = 8$). Groups with one or two asterisks are different ($P \leq 0.05$) from control and each other by analysis of variance and Fisher's protected least significant difference.

(5×10^6 CFU) or SRB51 (5×10^6 or 5×10^8 CFU) had significantly ($P \leq 0.05$) enhanced resistance to infection with S2308 at 12, 16, or 20 weeks after vaccination compared with nonvaccinated control mice (Fig. 2A). However, mice vaccinated with either 5×10^6 or 5×10^8 CFU of SRB51 had significantly ($P \leq 0.05$) lower resistance than did mice vaccinated with 5×10^6 CFU of S19. Spleen weights in S19-vaccinated mice were not significantly different from spleen weights in either of the two groups of SRB51-vaccinated mice following challenge of all vaccinated mice with S2308 (Fig. 2B). However, spleen weights in all groups of vaccinated mice following challenge with S2308 were significantly ($P \leq 0.05$) lower than the spleen weights in S2308-challenged, nonvaccinated control mice (Fig. 2B).

Serologic responses in vaccinated mice before and after challenge with S2308. Mice vaccinated with 5×10^6 CFU of S19 had significantly ($P \leq 0.01$) increased antibodies to S2308 bacteria (Fig. 3A) and S2308 smooth LPS (Fig. 3B) at 4, 8, and 12 weeks after vaccination compared with nonvaccinated control mice. In contrast, mice vaccinated with 5×10^6 CFU of SRB51 failed to produce antibody to either S2308 bacteria (Fig. 3A) or S2308 LPS (Fig. 3B) compared with nonvaccinated control mice. Similar results occurred when mice were vaccinated with a 100-fold-higher dose of SRB51 (5×10^8 CFU) in that they also failed to produce antibody to S2308 LPS (Fig. 3B). However, mice vaccinated with the 100-fold-higher dose of SRB51 had significant ($P \leq 0.01$) levels of antibody to S2308 bacteria at 4 and 8 weeks, but not at 12 weeks, after vaccination (Fig. 3A). Concentrations of antibodies to S2308 in these mice were significantly ($P \leq 0.01$) lower and less persistent, however, than those in mice vaccinated with S19. Mice vaccinated with S19 and then challenged with S2308 at 12, 16, or 20 weeks after vaccination had significantly ($P \leq 0.01$) higher antibody concentrations to S2308 bacteria (Fig. 3C) and S2308 smooth LPS (Fig. 3D) than did nonvaccinated control mice that had been challenged with S2308. However, antibodies to S2308 (Fig. 3C) and S2308 LPS (Fig. 3D) were not different among both groups of SRB51-vaccinated mice following challenge with S2308 compared with S2308-challenged, nonvaccinated control mice.

Spleen cell proliferation to S2308 or S2308 proteins in vaccinated mice following challenge with S2308. Mice vaccinated with S19 (5×10^6 CFU) or SRB51 (5×10^6 or 5×10^8 CFU) had significantly ($P \leq 0.05$) increased spleen cell proliferative responses to S2308 when they were challenged with S2308 at 12, 16, or 20 weeks after vaccination (Fig. 4). During these times, the responses in mice vaccinated with either dose of SRB51 were either the same or significantly ($P \leq 0.05$) lower than those in mice vaccinated with S19, depending on the concentration of S2308 which was incubated with the spleen cells.

Mice vaccinated with 5×10^6 CFU of SRB51 and then challenged 12 weeks later with S2308 had spleen cell proliferative responses to 106- to 18-kDa proteins of S2308 (Fig. 5C) that were similar to those in S2308-challenged, nonvaccinated control mice (Fig. 5A). In contrast, spleen cells from mice vaccinated with 5×10^6 CFU of S19 (Fig. 5B) or 5×10^8 CFU of SRB51 (Fig. 5D) and challenged at week 12 with S2308 exhibited higher proliferation to the S2308 proteins than did S2308-challenged, nonvaccinated control mice (Fig. 5A). In addition, similar proliferation responses to the 106- to 18-kDa proteins of S2308 were found in spleen cells from mice that had been vaccinated with 5×10^6 CFU of S19 or 5×10^8 CFU of SRB51. Results similar to those shown in Fig. 5 were obtained when spleen cells from mice vaccinated with S19 (5×10^6 CFU) or either dose of SRB51 (5×10^6 or 5×10^8 CFU) were incubated with the S2308 protein fractions following challenge of the mice with S2308 at 16 or 20 weeks after vaccination (data not shown).

DISCUSSION

Previous studies have not compared resistance to infection with S2308 in mice vaccinated with SRB51 or S19, although it has previously been noted that mice given 10^8 CFU of SRB51 have enhanced resistance to infection when challenged 7 weeks later with S2308 (15). In the current study, mice vaccinated

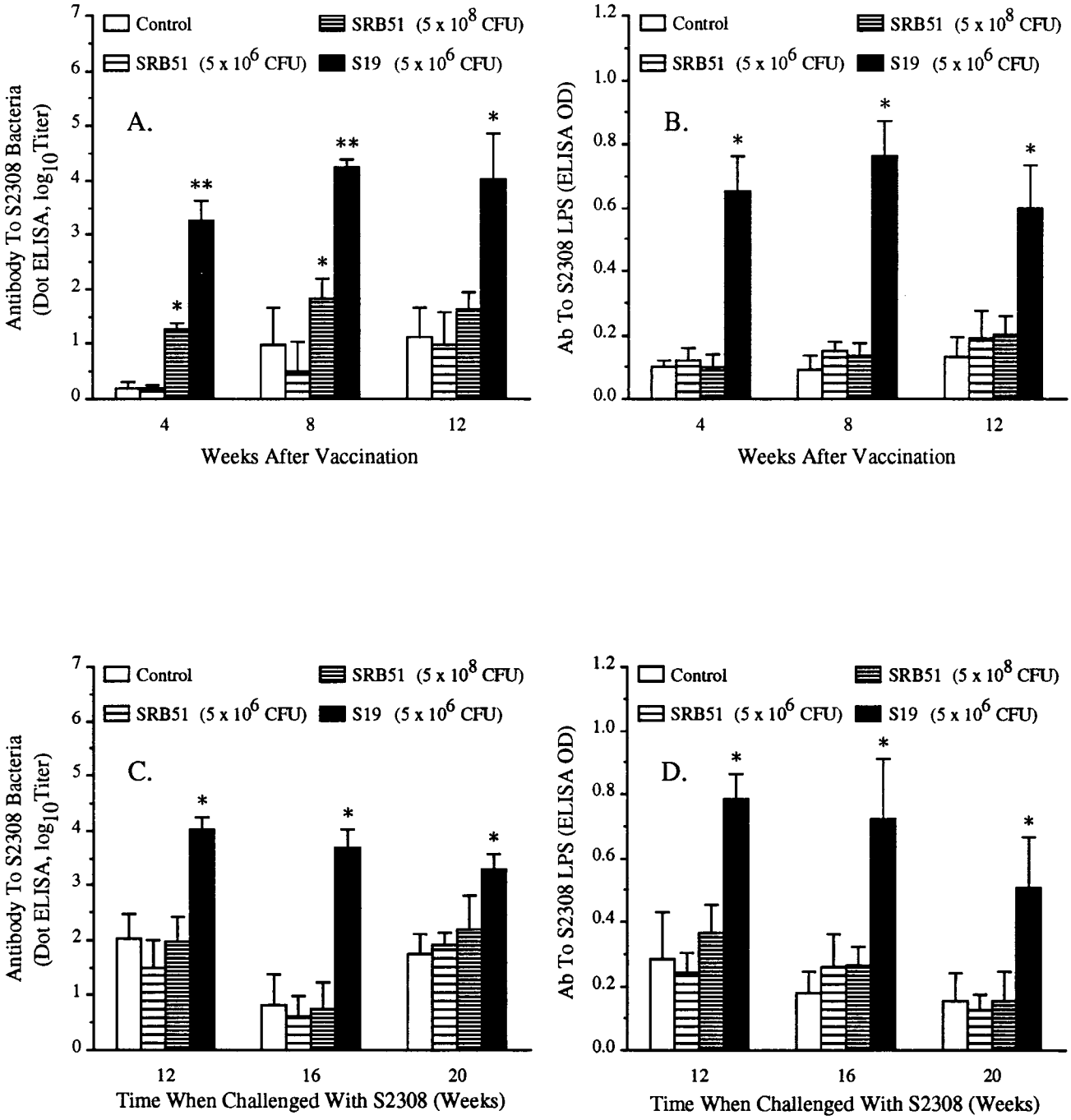

FIG. 3. Serologic responses in vaccinated mice before and after challenge with S2308. Mice were vaccinated with S19 (5×10^6 CFU) or SRB51 (5×10^6 or 5×10^8 CFU). Antibody to S2308 and S2308 LPS were measured in vaccinated mice at 4, 8, and 12 weeks (A and B) and in vaccinated mice at 2 weeks after challenge with S2308 at 12, 16, and 20 weeks (C and D). Results are expressed as mean ± SD ($n = 8$). Groups with one or two asterisks are different ($P \leq 0.01$) from controls and each other by analysis of variance and Fisher's protected least significant difference.

with 5×10^6 or 5×10^8 CFU of SRB51 had increased resistance to infection with S2308 at 12 to 20 weeks after vaccination. However, the resistance induced by either dose of SRB51 was lower than that induce by vaccinating mice with 5×10^6 CFU of S19. The lower resistance to infection probably resulted from a combination of SRB51 being less persistent and persisting in lower numbers following vaccination than did S19 and an absence of antibodies to S2308 smooth LPS in SRB51-vaccinated mice. Lower cell-mediated immune responses would be a less likely explanation for the lower resistance in mice given SRB51 because these mice and mice given S19 generally had similar spleen cell proliferative responses to S2308 bacteria and bacterial proteins (106 to 18 kDa) following infection with S2308.

Results from previous studies have indicated that mice given SRB51 produce antibodies to the outer membrane proteins (15) but not to the LPS O antigens of S2308 (15, 22). Data from the current study complement and extend these findings

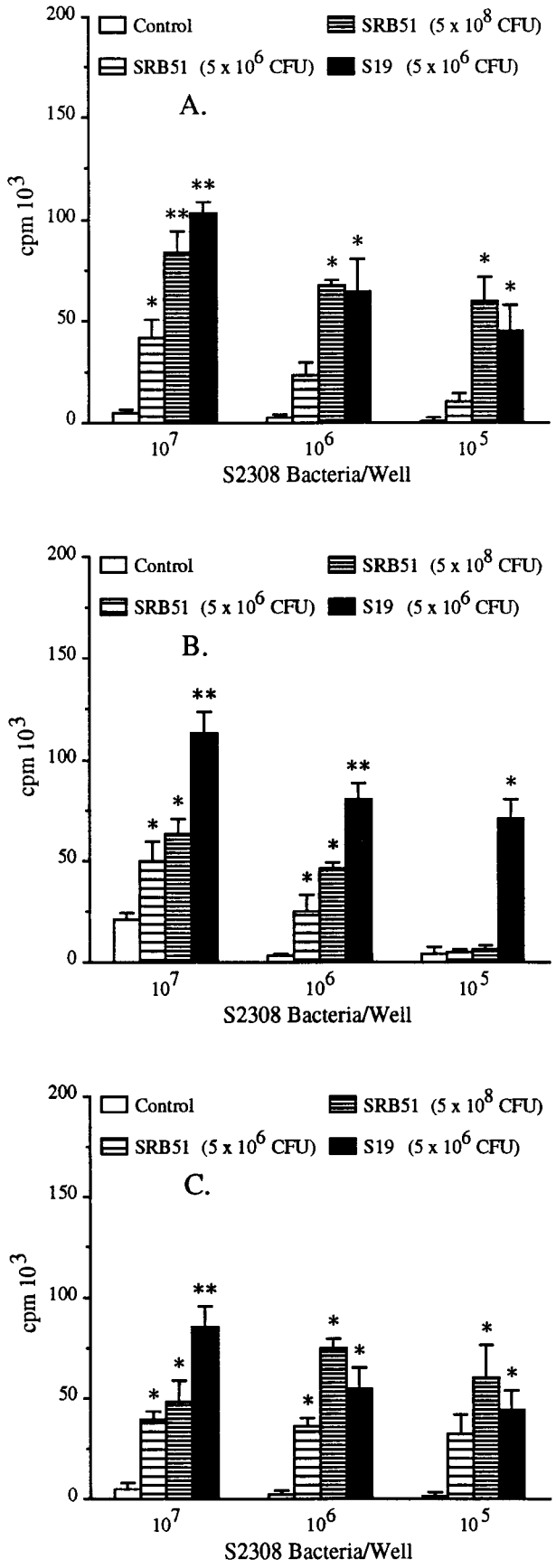

FIG. 4. Spleen cell proliferation to S2308 in vaccinated mice following challenge with S2308. Mice were challenged with S2308 at 12 (A), 16 (B), or 20 (C) weeks after vaccination with S19 (5×10^6 CFU) or SRB51 (5×10^6 or 5×10^8 CFU). Spleen cell suspensions were prepared from mice at 2 weeks after challenge. Cells (3×10^5 cells per well) were incubated for 5 days with 10^7 to 10^5 γ-irradiated killed S2308 and then pulsed for 18 h with [^{3}H]thymidine. Results are expressed as mean ± SEM ($n = 4$). Groups with one or two asterisks are different ($P \leq 0.05$) from controls and each other by analysis of variance and Fisher's protected least significant difference.

because SRB51-vaccinated mice had no measurable antibody to the S2308 smooth LPS molecule, yet they had antibody which reacted with the intact S2308 bacterium. Therefore, antibody in these mice probably reacted with the outer membrane proteins or other surface antigens of S2308 but not with components of S2308 LPS, including the O antigens, core region, or lipid A. Many studies using passive transfer experiments have demonstrated that antibodies to *B. abortus* smooth LPS have an important role in immunity to brucellosis in mice (1, 2, 5, 7, 8, 10, 24). In the current study, only S19-vaccinated mice produced antibody to S2308 smooth LPS, and this was probably a significant contributing factor to their higher resistance to infection with S2308 than occurred in SRB51-vaccinated mice.

Mice given 5×10^6 or 5×10^8 CFU of SRB51 had lower numbers of bacteria in the spleen and more rapid clearance of bacteria from the spleen than did mice given 5×10^6 CFU of S19. Thus, S19 persisted longer and in higher numbers in the spleen than did SRB51 even though mice were given approximately 100 times more SRB51. Increasing the vaccine dose of SRB51 did not appreciably increase the persistence of SRB51 in mice following vaccination. In addition, increasing the dose of SRB51 did not appreciably increase the spleen cell proliferative responses to S2308 bacteria following infection of the vaccinated mice with S2308. In fact, the single major difference between mice given the two different doses of SRB51 was that only mice given the highest dose had antibody that reacted to S2308 bacteria. The significance of these findings is not clear, however, because antibody titers in these mice were considerably lower and less persistent than those in mice vaccinated with S19, and antibodies to S2308 were absent at 12 weeks or later when the SRB51-vaccinated mice were challenged with S2308. Therefore, the low and short-term antibody responses to S2308 bacteria in mice vaccinated with SRB51 probably had no major role in conferring resistance to infection with S2308. Instead, immunity to infection with S2308 in these mice probably resulted from only cell-mediated immune responses.

Mice were challenged with S2308 at 12, 16, or 20 weeks after vaccination with SRB51 or S19. These challenges occurred when the vaccinated mice had no SRB51 or had fewer than 10 S19 bacteria in the spleen (12-week challenge) or when these strains had been absence from the spleen for 4 to 8 weeks before the mice were challenged with S2308 (16- and 20-week challenge). We previously reported that S19 and SRB51 antigens do not persist after these strains have been cleared from the spleens in mice and that antigens contained in live, but not dead, S19 and SRB51 probably maintain stimulation of the immune system (20). Therefore, it is unlikely that mice exhibited protection from infection with S2308 in the current study by vaccine-induced nonspecific immune or inflammatory responses, because no SRB51 and essentially no S19 occurred in the spleens of vaccinated mice to induce these types of responses during 4 to 8 weeks before they were challenged with S2308. Instead, our data indicated that protection resulted from vaccine-induced specific immune responses which oc-

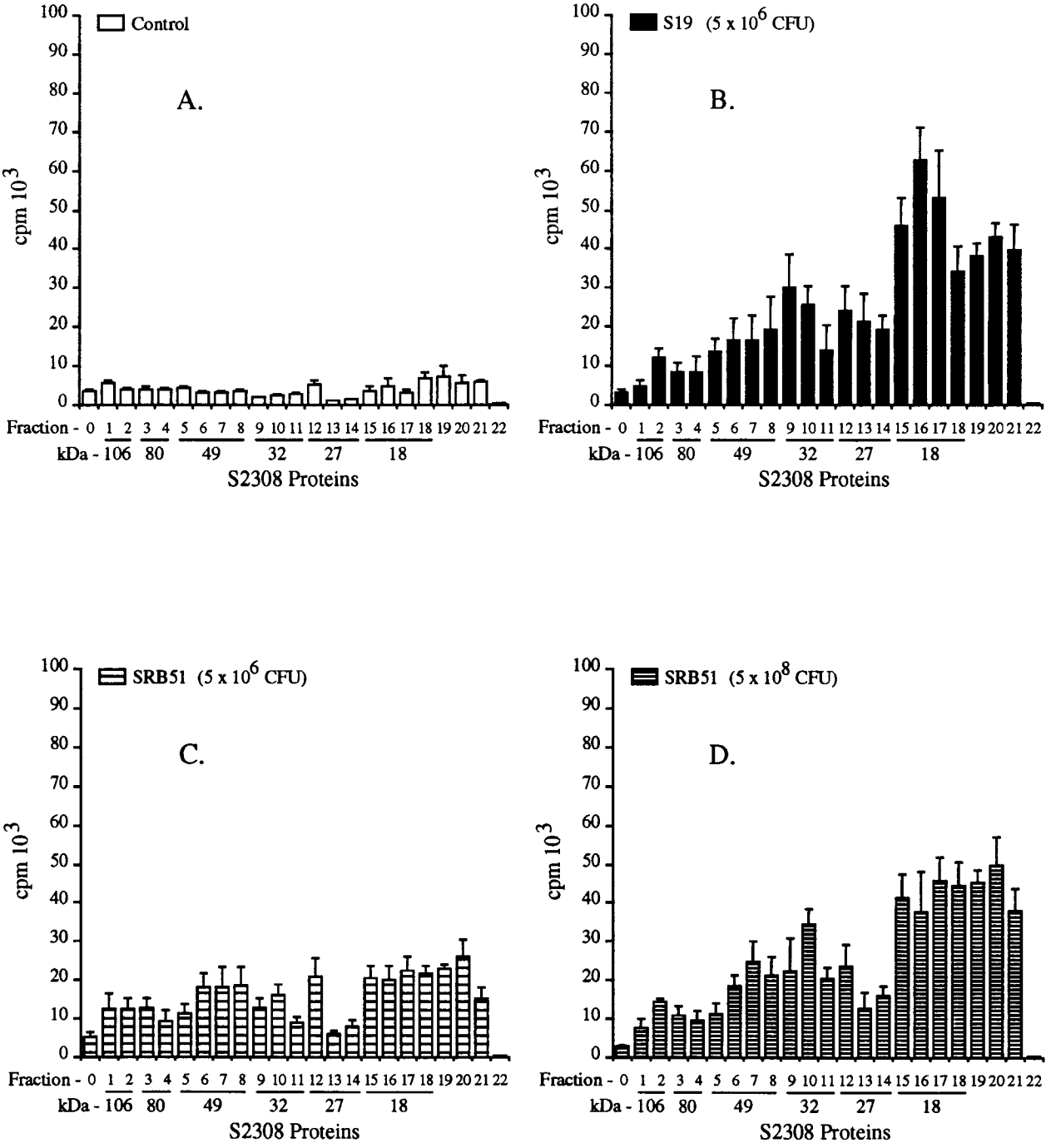

FIG. 5. Spleen cell proliferation to S2308 proteins in vaccinated mice following challenge with S2308. Mice were challenged with S2308 at 12 weeks after vaccination with S19 (5×10^6 CFU) or SRB51 (5×10^6 or 5×10^8 CFU). Spleen cells were obtained from nonvaccinated control mice (A) or from mice vaccinated with either S19 (5×10^6 CFU [B]) or SRB51 (5×10^6 [C] or 5×10^8 CFU [D]) at 2 weeks after challenge with S2308. Cells (3×10^5 cells per well) were incubated alone (fraction 0) or with S2308 proteins (fractions 1 to 22) for 5 days and then pulsed for 18 h with [^{3}H]thymidine. Results are expressed as mean ± SEM ($n = 4$).

curred after the vaccine strains were cleared from the vaccinated mice.

Results from the current study indicated that low persistence of SRB51 in vaccinated mice appears to be an inherent property of this bacterium which is not appreciably affected by altering the vaccine dose. The mechanism responsible for the rapid clearance of SRB51 is not known. However, we previously speculated that (i) reduced virulence and immunosuppressive activity of SRB51 and (ii) rapid ingestion and killing of SRB51 by macrophages may be contributing factors to its short survival when given to mice (20). In the present study, the highest tested dose of the SRB51 vaccine was 5×10^8 CFU, which is five times greater than that previously tested as a vaccine for preventing brucellosis in mice (15). Five $\times 10^8$ CFU of SRB51 is probably close to the maximum dose that can be tolerated by mice, because mice given this dose in the

current study were lethargic during the first 4 days following vaccination. Thus, higher doses of SRB51 than were used in the current study would probably not be beneficial in further increasing immune responses or resistance to infection with S2308 in mice.

Results from experiments that measure resistance to infection in vaccinated animals depend on many factors, including time after vaccination when animals are challenged, the challenge dose of the infecting organism, and time after challenge when resistance to infection is measured. The latter factor may have the most relevance to the current study because previous studies have noted that antibodies to S2308 smooth LPS enhance resistance and aid cell-mediated immunity during 1 to 2 weeks after infection of mice with S2308 (1, 5, 9, 24). Therefore, S19-vaccinated mice may have had higher resistance to infection than did SRB51-vaccinated mice in the current study, because only S19-vaccinated mice had antibody to S2308 LPS when mice were challenged with S2308. Consequently, mice vaccinated with SRB51 may have cleared S2308 more slowly because they had cell-mediated immune responses but no antibody responses to S2308. In view of these differences, we cannot preclude the possibility that mice vaccinated with SRB51 may have exhibited higher resistance if resistance had been measured for longer than 2 weeks after infection as used in this study. Studies comparing the temporal resistance to infection following challenge of S19-vaccinated or SRB51-vaccinated mice with S2308 may be required to fully assess the effectiveness of SRB51 in preventing brucellosis in mice.

We concluded from our previous studies that mice given SRB51 have lower and less persistent immune responses to S2308 than do mice given S19 (19, 20). As reported here, mice given SRB51 or S19 had equivalent cell-mediated immune responses to S2308 following S2308 infection, although mice given SRB51 had lower resistance to infection. However, these results cannot be extrapolated to mean that SRB51 would be less effective than S19 in protecting cattle from natural infections with field strains of *B. abortus*. This possibility can be assessed only by evaluating SRB51 as a vaccine in cattle under field conditions. Furthermore, even though SRB51 is being tested as a vaccine in cattle because it does not induce antibody to the LPS O antigens, it remains to be determined if this characteristic will also make SRB51 less effective than S19 in preventing brucellosis in cattle.

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