Effects of exogenous recombinant interleukin-12 on immune responses and protection against *Brucella abortus* in a murine model

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Abstract

This study determined if murine interleukin-12 (IL-12) would influence immunity in mice vaccinated with live or killed *Brucella abortus* strain RB51 (SRB51). Mice received live or γ-irradiated SRB51 bacteria alone, or with IL-12 (0.5 or 1.0 μg, 2× or 3×), whereas other mice received saline or IL-12 alone. Post-vaccination antibody responses to live or killed SRB51 and clearance of live SRB51 from splenic tissue were not influenced by IL-12 treatments. Mice were challenged at 12 weeks with 4×10^4 cfu of *B. abortus* strain 2308 (S2308) and were euthanized 2 weeks later. The highest IL-12 treatment increased ($P < 0.05$) post-challenge antibody responses when co-administered with killed SRB51. Co-administration of 1.0 μg of IL-12 with live SRB51, but not killed SRB51, reduced ($P < 0.05$) S2308 colonization of splenic tissues. Our data suggest that although IL-12 may augment protective immunity induced by live SRB51, it does not influence protection induced by vaccination with killed SRB51.

Résumé

Afin de déterminer si l'interleukine-12 (IL-12) murine influencerait l'immunité de souris vaccinées avec la souche vivante ou tuée de Brucella abortus *RB51 (SRB51), des souris reçurent seulement des bactéries SRB51 vivantes ou irradiées, ou des bactéries avec IL-12 (0,5 ou 1,0 μg, 2 fois ou 3 fois), alors que d'autres souris ne reçurent que de la saline ou IL-12. Le niveau d'anticorps post-vaccinal au vaccin vivant ou tué de même que l'élimination de SRB51 vivant du tissu splénique ne furent pas influencés par les traitements à l'IL-12. Les souris furent soumises à une infection défi avec* 4×10^4 *ufc de* B. abortus *souche 2308 (S2308) et euthanasiées 2 semaines plus tard. Le traitement avec la dose la plus élevée d'IL-12, lorsque administré conjointement avec SRB51 tué, augmenta significativement* ($P < 0,05$) *la réponse post-infection en anticorps. L'administration conjointe de 1,0 μg d'IL-12 avec SRB51 vivant réduisit de manière significative* ($P < 0,05$) *la colonisation des tissus spléniques par S2308. Les résultats suggèrent que bien qu'IL-12 puisse augmenter l'immunité protectrice induite par SRB51 vivant, il n'influence pas la protection conférée par une vaccination avec SRB51.*

(Traduit par Docteur Serge Messier)

Introduction

Interleukin-12 (IL-12) is a cytokine that supports the differentiation of Th1 cells and has inhibitory effects on cytokines that promote Th2 cell development and proliferation (1,2). Due to its stimulatory effect on cellular immunity, exogenous IL-12 has been evaluated for beneficial effects in protecting against pathogens in which cellular immune responses are of importance. In murine models for *Listeria monocytogenes* (3) and *Leishmania major* (4), IL-12 had beneficial adjuvant effects on protective responses when combined with killed vaccines.

In general, killed brucellosis vaccines do not sufficiently induce cellular immune responses and are not as efficacious as live vaccines in cattle and other animals (5). Identification of adjuvants that stimulate protective cellular immunity may be required to develop a suitable non-living brucellosis vaccine. A non-living brucellosis vaccine would be desirable as it would eliminate the pathogenic problems that can be caused by the live bacteria that are in the currently available brucellosis vaccines. In a previous study, we found that a single administration of 0.5 μg of recombinant murine IL-12 at the time of vaccination with live or killed *Brucella abortus* strain RB51 (SRB51), did not influence immune responses, clearance of live SRB51, or protection against challenge with the virulent 2308 strain of *B. abortus* (S2308) (6). Although a single 0.5-μg administration of IL-12 did not influence protective immune responses, we hypothesized that higher or multiple dosages of exogenous IL-12 might have beneficial effects. Therefore, we conducted the study reported here, in which the effects of multiple dosages of 0.5 or 1.0 μg of recombinant IL-12 on immune responses were evaluated when administered with live or killed SRB51 in a murine model of brucellosis.

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Table I. Serologic responses at 4 wk after IP vaccination of mice with live or killed *Brucella abortus* strain RB51 (SRB51) administered with, or without, recombinant interleukin-12 (IL-12)

Treatment	Log_{10} antibody titer to SRB51		
	IgG1	IgG2a	IgG2b
Saline	0.00 ± 0.00	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Live SRB51	1.07 ± 0.53	2.61 ± 0.10[b]	0.97 ± 0.49[b]
Live SRB51 + 0.5 μg IL-12 (3×)	0.43 ± 0.43	2.61 ± 0.20[b]	0.00 ± 0.00[a]
Live SRB51 + 1.0 μg IL-12 (3×)	1.20 ± 0.42	2.43 ± 0.75[b]	1.45 ± 0.15[b]
Killed SRB51 + 1.0 μg IL-12 (2×)	0.73 ± 0.42	1.98 ± 0.26[b]	0.98 ± 0.33[b]
Killed SRB51 + 1.0 μg IL-12 (3×)	1.70 ± 0.20	0.43 ± 0.43[a]	2.40 ± 0.27[c]

Mice (n = 4/treatment) were given 3×10^8 cfu of live or killed vaccine alone, or combined with 0.5 or 1.0 μg of recombinant murine IL-12 administered 2× (days 0 and 5) or 3× (days 0, 5, and 21). Other mice were injected IP with 0.2 mL of saline alone

Results are expressed as log_{10} mean dot-blot titer ± SEM

[a,b,c] Means with different superscripts within an antibody isotype are statistically different ($P \leq 0.05$)

Materials and methods

Cell culture

All cell culture experiments used RPMI 1640 medium (GIBCO Laboratories, Grand Island, New York, USA) containing l-glutamine, 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 10% fetal bovine serum (Hyclone Laboratories, Logan, Utah, USA), 100 U of penicillin per mL, 100 μg of streptomycin per mL, and 5×10^{-5} M 2-mercaptoethanol (RPMI).

Bacterial cultures

Live cultures of the S2308 challenge strain and the SRB51 vaccine strain were prepared as described previously (7). Killed cultures of SRB51 and S2308 were prepared by γ-irradiation with 1.4×10^6 rads. Recombinant murine IL-12 (IL-12) was used (R & D Systems, Minneapolis, Minnesota, USA).

Animals and inoculation

Female 10-week-old BALB/c AnNHsD mice (Harlan Sprague Dawley, Indianapolis, Indiana, USA) were used in the experiments. Mice (n = 12/treatment (Tx)) were vaccinated intraperitoneally (IP) with 0.2 mL of saline containing 3×10^8 colony-forming units (cfu) of live SRB51 alone (live SRB51), or combined with 0.5 or 1.0 μg of murine recombinant IL-12, administered 3× (days 0, 5, and 21, (3×)). Additional mice were vaccinated IP with 0.2 mL of saline containing 3×10^8 cfu of γ-irradiated SRB51 alone (killed SRB51, n = 8), or combined with 0.5 (n = 8/Tx) or 1.0 μg of IL-12 (n = 12/Tx) administered 2× (days 0 and 5) or 3×. Other mice were injected IP with 0.2 mL of saline alone (saline, n = 12) or saline containing 0.5 or 1.0 μg of IL-12 administered 2× or 3× (n = 8/Tx).

Four mice in saline control, live SRB51, live SRB51 + 0.5 μg IL-12 (3×), live SRB51 + 1.0 μg IL-12 (3×), and killed SRB51 + 1.0 μg IL-12 (2× and 3×) treatments were euthanized with CO_2/O_2 at 4 wk after inoculation. Blood samples, spleens, and livers were obtained at necropsy. Blood was allowed to clot for 12 h at 4°C, centrifuged, and serum stored at −70°C until use. Spleens and livers were weighed and stored at −69°C until processed for bacteriologic evaluation.

Remaining mice (n = 8/trt) were challenged IP with 4×10^4 cfu of S2308 at 12 wk after vaccination. The time of challenge was based on data from previous studies which demonstrated that a dosage of 5×10^8 cfu of SRB51 is cleared from BALB/c mice by 12 wk after IP vaccination (7). Following euthanasia with CO_2/O_2 at 2 wk after S2308 challenge, blood, spleens, and livers were obtained from all mice. Spleens were weighed, approximately one-half was excised for bacteriologic examination and weighed, and remaining tissue was used to prepare spleen cell suspensions. Following weighing, the entire liver was designated for bacteriologic examination. Tissue for bacteriologic examination was stored at −69°C until processed.

Cell proliferation assays

The portion of the spleen for use in proliferation assays was placed on a sterile 60-mesh stainless steel screen, minced with scissors, and processed to form spleen cell suspensions, as described previously (7). After S2308 challenge, spleen cell suspensions from 8 mice in each treatment were combined into 4 suspensions (2 mice/suspension, 4 suspensions/treatment) for evaluation of lymphocyte proliferative responses. Briefly, 50 μL of RPMI containing 3×10^5 spleen cells was added to each of 2 separate, flat-bottomed wells of a 96-well microtiter plate that contained 100 μL of γ-irradiated SRB51 or S2308 (10^8, 10^7, 10^6, 10^5, and 0 bacteria per well). Cell cultures were incubated for 5 d at 37°C in 5% CO_2 and pulsed for an additional 18 h with 1.0 μCi of [^{3}H] thymidine per well. Cells were then harvested (Harvester 96; Tomtec Inc., Orange, Connecticut, USA) and measured for radioactivity in a liquid scintillation counter (1450 Microbeta; Wallace Inc., Gaithersburg, Maryland, USA). Cell proliferation results were expressed as stimulation indices (counts per minute in presence of antigen/counts per minute in media only).

Serology

Serum samples were measured for antibodies to SRB51 after vaccination, or S2308 after challenge, by a previously described dot enzyme-linked immunosorbent assay (ELISA) procedure (8), using 1:2000 dilutions of goat anti-mouse IgG1, goat anti-mouse IgG2a, or

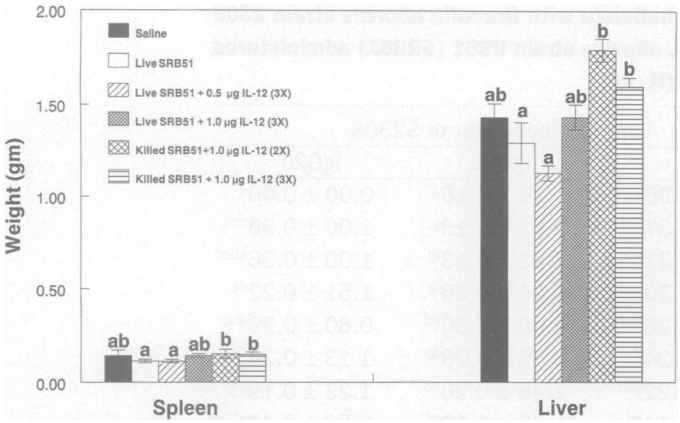

Figure 1. Weights of splenic and hepatic tissues at 4 weeks after vaccination with 3 × 10^8 cfu of live or killed SRB51 alone, or combined with 0.5 or 1.0 µg of murine recombinant IL-12 administered on days 0 and 5 (2×), or days 0, 5, and 21 (3×) (n = 4 mice/treatment). [a,b] Means with different superscripts differ significantly ($P < 0.05$) for that tissue.

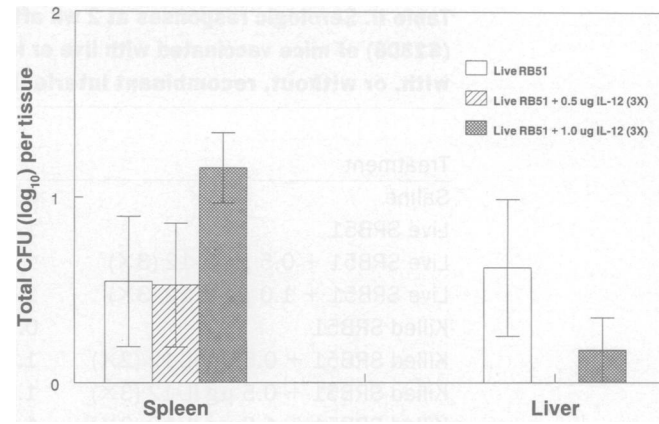

Figure 2. Colonization of splenic and hepatic tissues (mean log Total cfu per tissue ± SEM) 4 wk after vaccination with 3 × 10^8 cfu of SRB51 alone, or combined with 0.5 or 1.0 µg of IL-12 administered on days 0, 5, and 21 (3×) (n = 4 mice/treatment).

goat anti-mouse IgG2b conjugated with horseradish peroxidase (Bethyl Laboratories Inc., Montgomery, Texas, USA). Results from the dot ELISA were expressed as mean $\log_{10}$ titer ± standard error of the mean (SEM).

Bacteriologic procedures

The portions of spleens and livers retained for bacteriologic evaluation were processed in a tissue grinder to form a cell lysate, serially diluted in saline, and placed on tryptose agar plates containing 5% bovine serum (TSA). After incubation of plates at 37°C with 5% CO_2 for 72 h, bacterial cell counts were made from each dilution by standard plate counts. *Brucella* spp. isolates were identified based on colony morphology, growth characteristics (9), and a *Brucella* spp.-specific polymerase chain reaction (PCR) procedure. Strain RB51 was also identified based on rifampin resistance (10). Briefly, the PCR reaction mixture consisted of heat-killed cells, 0.2 mM nucleotide mix (Boehringer Mannheim, Mannheim, Germany), 1X PCR buffer II (Perkin Elmer, Branchburg, New Jersey, USA), 2.5 U/mL DNA polymerase (Amplitaq Gold; Perkin Elmer), and 1.5 mM MgCl combined with 20 pm per reaction of specific primers for *B. abortus* omp2A, as selected from analysis of its sequence (11) (upstream primer GCAACGGTGTTCTTCCACTC and downstream primer GTATCAGGCTACGCAGAAGG). The samples, including positive and negative controls, were cycled (30 s at 95°C, 30 s at 44°C, 1 min at 72°C) 30 times in a thermocycler (MJ Research Inc., Watertown, Massachusetts, USA). Products (5 to 8 µL from each reaction mixture) were analyzed by electrophoresis on a 1.5% agarose gel after staining with ethidium bromide. Results were then expressed as mean $\log_{10}$ cfu per g ± SEM, or as mean $\log_{10}$ cfu per total spleen or liver (cfu per g × total weight).

Statistical Analysis

For statistical comparisons, spleen weight, liver weight, dot-blot titers, and colonization data (cfu/g and total cfu/tissue) were analyzed as the logarithm of their value. Due to conversion to logarithm, titers or colonization data with a value of 0 were analyzed with a value of 1. Statistical differences between treatments were determined by an analysis of variance procedure, and significant differences were reported when $P \leq 0.05$. Means for each treatment were compared by use of a least significant difference procedure and are reported as least square means ± SEM.

Results

At 4 wk after inoculation, mice inoculated with saline had no detectable IgG1, IgG2a, or IgG2b antibody responses to SRB51 (Table I). At this sampling time, IgG1 antibody responses were not influenced ($P > 0.05$) by vaccination treatment. With the exception of IgG2b responses of mice inoculated with live SRB51 + 0.5 µg IL-12 (3×), and IgG2a responses of mice inoculated with killed SRB51 + 1.0 µg IL-12 (3×), mice inoculated with live or killed SRB51, with or without co-administration of IL-12, had greater ($P < 0.05$) IgG2a and IgG2b antibody responses to SRB51 at 4 wk after inoculation when compared with responses of mice inoculated with saline. Mice in the killed SRB51 + 1.0 µg IL-12 (3×) treatment had antibody responses which were predominantly IgG2b, whereas mice receiving live SRB51, with or without exogenous IL-12, tended to have greater IgG2a antibody responses. Mice receiving killed SRB51 + 0.5 µg IL-12 (3×) also tended to have greater IgG2a antibody responses.

At 4 wk after inoculation, spleens and livers of mice inoculated with live SRB51 + 0.5 µg IL-12 (3×) weighed less ($P < 0.05$) when compared with spleens and livers of mice inoculated with killed SRB51 + 1.0 µg IL-12 (2× or 3×) (Figure 1). Interleukin-12 did not enhance splenic or hepatic clearance of live SRB51 at 4 wk after inoculation as tissue colonization (cfu/gm and total cfu) did not differ ($P > 0.05$) between mice inoculated with live SRB51 alone, or when combined with 3× administration of 0.5 or 1.0 µg of IL-12 (Figure 2).

At 2 wk following S2308 challenge, mice vaccinated with live SRB51, with or without 0.5 or 1.0 µg of IL-12 (3×), tended to have greater IgG2a dot-blot titers against S2308 when compared to IgG2b responses (Table II). Although co-administration of 0.5 µg of IL-12 (3×) with live SRB51 reduced ($P < 0.05$) IgG2a responses when compared with live SRB51 alone, antibody responses to S2308 by mice in other live SRB51 treatments were not influenced by exogenous IL-12 (Table II). Administration of 0.5 or 1.0 µg of IL-12 to mice

Table II. Serologic responses at 2 wk after IP challenge with *Brucella abortus* strain 2308 (S2308) of mice vaccinated with live or killed *B. abortus* strain RB51 (SRB51) administered with, or without, recombinant interleukin-12 (IL-12)

Treatment	Log_{10} antibody titer to S2308		
	IgG1	IgG2a	IgG2b
Saline	0.53 ± 0.26^{ab}	0.16 ± 0.16^{a}	0.00 ± 0.00^{a}
Live SRB51	1.00 ± 0.37^{bcd}	2.63 ± 0.13^{g}	1.00 ± 0.36^{bcd}
Live SRB51 + 0.5 μg IL-12 (3×)	1.00 ± 0.31^{bcd}	1.93 ± 0.13^{ef}	1.00 ± 0.36^{abcd}
Live SRB51 + 1.0 μg IL-12 (3×)	1.00 ± 0.30^{bcd}	2.24 ± 0.26^{fg}	1.51 ± 0.22^{de}
Killed SRB51	0.73 ± 0.28^{abcd}	1.00 ± 0.30^{bd}	0.60 ± 0.29^{abc}
Killed SRB51 + 0.5 μg IL-12 (2×)	1.51 ± 0.24^{d}	0.56 ± 0.28^{ab}	1.13 ± 0.26^{cd}
Killed SRB51 + 0.5 μg IL-12 (3×)	1.08 ± 0.22^{bcd}	1.48 ± 0.20^{cd}	1.23 ± 0.19^{cde}
Killed SRB51 + 1.0 μg IL-12 (2×)	1.53 ± 0.14^{cd}	1.75 ± 0.19^{df}	0.80 ± 0.48^{abcd}
Killed SRB51 + 1.0 μg IL-12 (3×)	1.54 ± 0.36^{d}	2.05 ± 0.13^{efg}	1.90 ± 0.22^{e}
0.5 μg IL-12 (2×)	0.19 ± 0.19^{a}	0.97 ± 0.25^{bcd}	0.60 ± 0.29^{abc}
0.5 μg IL-12 (3×)	0.93 ± 0.28^{acd}	1.08 ± 0.33^{bcd}	1.16 ± 0.27^{cde}
1.0 μg IL-12 (2×)	0.49 ± 0.24^{ab}	0.89 ± 0.26^{bc}	0.33 ± 0.21^{ab}
1.0 μg IL-12 (3×)	0.84 ± 0.33^{acd}	1.39 ± 0.33^{cde}	1.07 ± 0.33^{cd}

Mice were given 3×10^8 cfu of live or killed vaccine alone, or combined with 0.5 or 1.0 μg of recombinant murine IL-12 administered 2× (days 0 and 5) or 3× (days 0, 5 and 21). Other mice were injected IP with 0.2 mL of saline alone or saline containing 0.5 or 1.0 μg of IL-12 administered 2× or 3×. Mice (n = 8/treatment) were challenged with 4×10^4 cfu of S2308 at 12 wk after vaccination Results are expressed as mean dot-blot titer (log_{10}) ± SEM

[a,b,c,d] Means with different superscripts within an antibody isotype are statistically different ($P \leq 0.05$)

inoculated with killed SRB51 tended to enhance the magnitude of IgG1, IgG2a, and IgG2b antibody responses to S2308 as compared to killed SRB51 alone, with significant increases ($P < 0.05$) in IgG2a and IgG2b titers in mice receiving 3× administrations of the highest IL-12 dosage (1.0 μg). Combining 0.5 or 1.0 μg of IL-12 with killed SRB51 had no definitive effect in shifting antibody isotype responses to S2308 antigens. Post-challenge IgG2a antibody responses in mice inoculated with IL-12 only (0.5 or 1.0 μg, 2× or 3×) were greater ($P < 0.05$) than responses of mice inoculated with saline.

Post-challenge proliferative responses to S2308 by spleen cells from mice inoculated with live or killed SRB51 were not influenced ($P > 0.05$) by 2× or 3× administration of 0.5 or 1.0 μg of IL-12 (data not shown).

Mice inoculated with live SRB51, with or without 0.5 or 1.0 μg of IL-12, had lower ($P < 0.05$) spleen weights at 2 wk after S2308 challenge (Figure 3A) when compared to mice inoculated with saline or killed SRB51 alone. At necropsy after challenge, liver weights of mice inoculated with live SRB51 + 1.0 μg IL-12 (3×) were lower ($P \leq 0.05$) when compared to liver weights of mice inoculated with saline or killed SRB51 alone. Administration of 0.5 or 1.0 μg of IL-12 to mice inoculated with killed SRB51 did not reduce ($P > 0.05$) spleen or liver weights after S2308 challenge (Figure 3B) when compared to mice inoculated with killed SRB51 alone.

Mice inoculated with live SRB51 had reduced ($P < 0.05$) S2308 colonization (total cfu and cfu/g) of spleen and liver at 2 wk after challenge when compared to mice inoculated with saline (Figure 4A). In a similar manner, mice inoculated with live SRB51 had fewer ($P < 0.05$) cfu of S2308 per g of splenic tissue and reduced ($P < 0.05$) S2308 colonization (total cfu and cfu/g) of hepatic tissue when compared to killed SRB51. Co-administration of 0.5 or 1.0 μg of IL-12 (3×) with live SRB51 reduced ($P < 0.05$) total cfu of S2308 per g of splenic tissue but did not influence ($P > 0.05$) S2308 colonization of hepatic tissue when compared to mice inoculated with live SRB51 alone. When compared to mice inoculated with killed SRB51 only, co-administration of 0.5 or 1.0 μg of IL-12 (2× or 3×) with killed SRB51 did not reduce splenic colonization ($P > 0.05$) and, with the exception of 1.0 μg of IL-12 (3×), did not influence ($P > 0.05$) colonization of hepatic tissues (Figure 4B). Mice inoculated with 0.5 or 1.0 μg of IL-12 alone (2× or 3×) had reduced ($P < 0.05$) S2308 colonization (total cfu and cfu/g) in hepatic tissues, but not splenic tissues, when compared to mice inoculated with saline or killed SRB51.

Discussion

The current study was designed to determine if repeated administrations of IL-12, or dosages as high as 1.0 μg, would modify immune responses to live or killed SRB51, or enhance resistance to experimental *Brucella* challenge. Others have found that IL-12 augments protective immunity against *Listeria* (0.5 μg, days 0 and 5) or *Leishmania* (1.0 μg, days 0 and 10) when combined with killed *Listeria* or *Leishmania* only (3,4). Although it has been demonstrated that IL-12 plays a role in murine resistance to *B. abortus* (12,13), data from a previous study in our laboratory indicated that a single administration of 0.5 μg of IL-12 at the time of vaccination did not enhance immune responses to live or killed SRB51 (6). The data from the study reported here indicates that combining live SRB51 with 0.5 or 1.0 μg of IL-12 (days 0, 5, and 21) enhances protective immunity against a virulent strain of *B. abortus*.

The induction of γ-interferon production, either by infection with *B. abortus* (14,15), or via endogenous IL-12 production (13), has

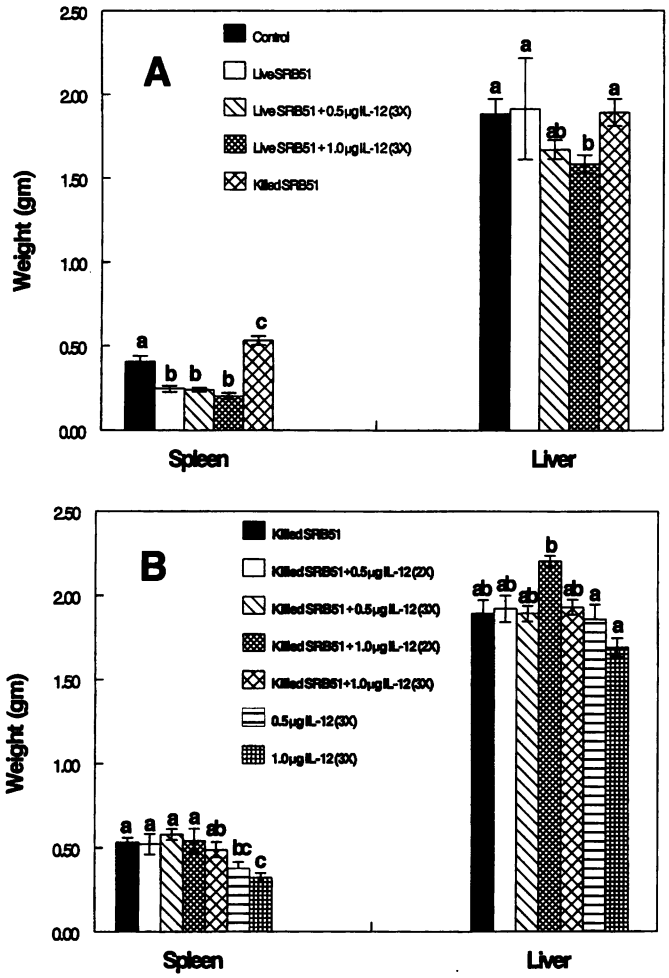

Figure 3. Weights of splenic and hepatic tissues at 2 wk after IP challenge with 4 × 10^4 cfu of S2308. Mice had been vaccinated 12 wk prior to challenge with live or killed SRB51, alone, or in combination with 0.5 or 1.0 μg recombinant murine IL-12 (2× or 3×), or, with saline or IL-12 alone (n = 8 mice/treatment). ^{a,b,c} Means with different superscripts differ significantly ($P < 0.05$) for that tissue.

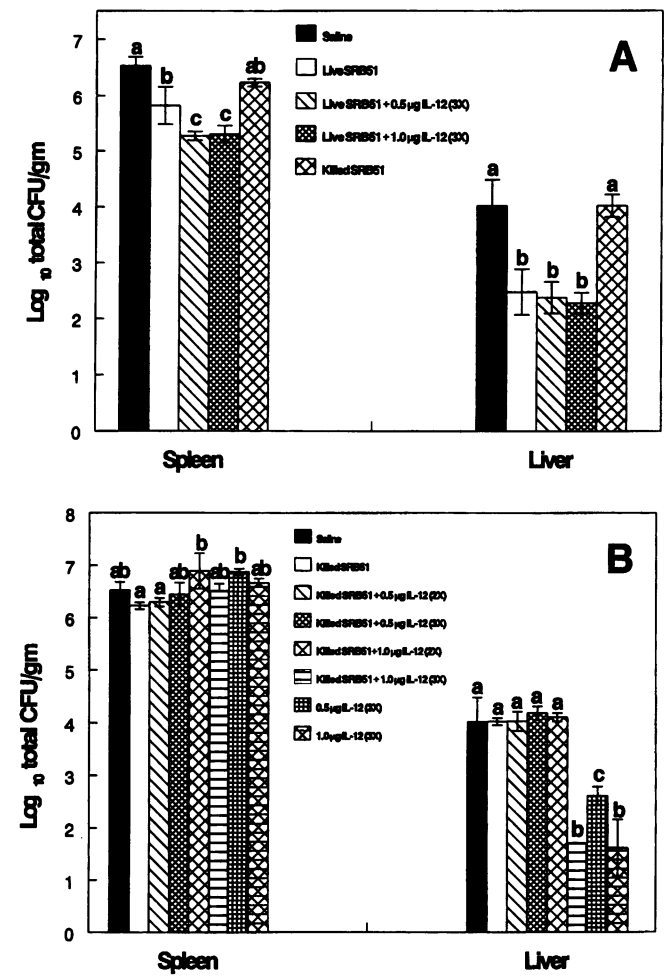

Figure 4. Colonization of splenic and hepatic tissues (mean total cfu (log_{10}) per tissue ± SEM) at 2 weeks after intraperitoneal challenge with 4 × 10^4 cfu of S2308. Mice had been vaccinated 12 wk prior to challenge with live or killed SRB51, alone, or in combination with 0.5 or 1.0 μg recombinant murine IL-12 (2× or 3×), or, with saline or IL-12 alone (n = 8 mice/treatment). ^{a,b,c} Means with different superscripts differ significantly ($P < 0.05$) for that tissue.

been associated with murine resistance to *Brucella* infection. This is also supported by data that demonstrated increased resistance in mice administered recombinant γ-interferon before, and during, infection with *B. abortus* (16), and decreased resistance in mice treated with an anti-IFN-γ monoclonal antibody prior to infection (17). It has been demonstrated that IL-12 stimulates increased γ-interferon production (18), which induces murine B cells to switch to IgG2a isotypes (19). In contrast, IL-4 production, which can be inhibited by IL-12 (18), is associated with a Th2 response and increased IgG1 antibody responses in mice (20). The augmented post-challenge IgG1, IgG2a, and IgG2b antibody responses induced by the highest concentration of IL-12 in our study suggest that combining exogenous IL-12 with killed SRB51 stimulated both Th1 and Th2 responses in response to S2308 infection. As Th1 responses are associated with resistance to infection with *Brucella*, the dichotomy of the antibody response may suggest the possibility that the exogenous IL-12 dosages used in the study reported here failed to stimulate sufficient cell-mediated immune responses to killed SRB51. This hypothesis is supported by failure of IL-12 to enhance post-challenge proliferative responses in mice inoculated with killed SRB51. As data from a passive transfer experiment suggested that antibody responses are not protective in murine models of *B. abortus* (21), the IL-12-induced increases in antibody responses to killed SRB51 noted in the present study would not be expected to have a positive correlation with increased resistance to S2308 challenge. Others have noted that IL-12 does not induce long lasting suppression of Th2 development (22). Although recall responses of mice inoculated with IL-12 and antigen can mimic the Th1 phenotype noted after immunization, the development of Th2 recall responses may also be supported (22).

Although not statistically different, there was a trend for the highest dosage of IL-12 to enhance proliferative responses to S2308 at 2 wk after challenge in mice inoculated with live SRB51. The increase in resistance to S2308 challenge in these mice may reflect this trend. Others have hypothesized that *B. abortus* interferes with immune responses by stimulating mainly the non-immunoactive IL-12p40 and down regulating secretion of the bioactive form, IL-12p70 (23). Data from our study indicate that exogenous IL-12 may

only partially potentiate immune responses induced by live *B. abortus* and do not eliminate the possibility that the IL-12 dosage regimens used were insufficient for maximal effects on immune responses. Although live S2308, but not purified lipopolysaccharide (LPS) from S2308, inhibits secretion of bioactive IL-12p70, both live S2308 and its LPS induce strong stimulation of γ-interferon from peritoneal macrophages (23). However, spleen cells from mice depleted of IL-12 before, or up to 7 d after infection with *B. abortus* strain 19, have reduced production of γ-interferon at 2 wk after infection (15). Therefore, if induction of γ-interferon synthesis is critical for protection to *Brucella* spp, in vivo activity of IL-12 is required for γ-interferon production but exogenous IL-12 may be of minimal benefit if live bacteria alone can stimulate secretion of high levels of γ-interferon.

Data may suggest that the bioactivity of IL-12 is most critical during acute stages of infection with *Brucella abortus* as administration of anti-IL-12 monclonal antibodies to mice at 10 wk after infection have no effect on λ-interferon secretion by spleen cells (11). In addition, it has been proposed that IL-12 mediates macrophage migration into murine splenic tissues and subsequent activation in early responses to *Brucella* infection (12,13,15).

The results of this study suggest that although higher dosages of IL-12 may augment protective immunity induced by vaccination with live SRB51, addition of exogenous IL-12 to a killed SRB51 vaccine does not influence resistance to *Brucella* challenge. Despite beneficial effects on efficacy when used with other bacterial species, our data suggests that IL-12 may not be an effective adjuvant for use with a killed *Brucella* vaccine in a murine model of brucellosis.

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