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Oral Immunization with an Attenuated Vaccine Strain of *Salmonella typhimurium* Expressing the Serine-Rich *Entamoeba histolytica* Protein Induces an Antiamebic Immune Response and Protects Gerbils from Amebic Liver Abscess

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Attenuated salmonellae represent attractive candidates for the delivery of foreign antigens by oral vaccination. In this report, we describe the high-level expression of a recombinant fusion protein containing the serine-rich *Entamoeba histolytica* protein (SREHP), a protective antigen derived from virulent amebae, and a bacterially derived maltose-binding protein (MBP) in an attenuated strain of *Salmonella typhimurium*. Mice and gerbils immunized with *S. typhimurium* expressing SREHP-MBP produced mucosal immunoglobulin A antiamebic antibodies and serum immunoglobulin G antiamebic antibodies. Gerbils vaccinated with *S. typhimurium* SREHP-MBP were protected against amebic liver abscess, the most common extraintestinal complication of amebiasis. Our findings indicate that the induction of mucosal and immune responses to the amebic SREHP antigen is dependent on the level of SREHP-MBP expression in *S. typhimurium* and establish that oral vaccination with SREHP can produce protective immunity to invasive amebiasis.

Entamoeba histolytica is an intestinal protozoan parasite that causes amebic dysentery and amebic liver abscess, diseases associated with significant morbidity and mortality worldwide. There is epidemiologic evidence to suggest that some degree of immunity to recurrent bouts of amebic liver abscess may be acquired after infection (3), and data from animal studies indicate that protective immunity to amebiasis can be induced by prior infection or vaccination (for a review, see reference 7). Recent studies using gerbil models of amebic liver abscess have established that parenteral vaccination with recombinant *E. histolytica* antigens can protect animals from invasive amebiasis (11, 12, 20, 22; for a review, see reference 14). One promising vaccine candidate has been the serine-rich *E. histolytica* protein (SREHP), a highly immunogenic surface antigen of amebic trophozoites (15, 17, 18). In three trials, parenteral immunization with SREHP as a maltose-binding protein (MBP) fusion protein protected 64 to 100% of vaccinated gerbils from amebic liver abscess (20, 22). Further evidence for the protective efficacy of SREHP vaccination has come from experiments using severe combined immunodeficient mice in which passive immunization with anti-SREHP serum was effective in preventing amebic liver abscess in the severe combined immunodeficient mouse model of disease (19).

While parenteral immunization with SREHP-MBP has proven effective in preventing amebic liver abscess in animal models of disease, there are a number of potential advantages to utilizing oral vaccination for protection against amebiasis. Amebiasis begins as an intestinal infection, and the stimulation of mucosal immune responses by oral immunization could potentially block the establishment of amebic infection. An oral formulation could have cost and ease-of-administration advantages over a parenteral vaccine. Finally, by expressing protective recombinant amebic antigens in attenuated vaccine strains of *Vibrio cholerae* or *Salmonella typhi*, one could possibly create a cost-effective combination vaccine which would be particularly useful at a time of globally scarce resources for health care.

As a step towards the development of an oral vaccine to prevent amebiasis, we previously expressed the SREHP-MBP molecule in an attenuated vaccine strain of *Salmonella typhimurium* and demonstrated that this strain could infect and colonize the gerbil, an animal used for experimental amebiasis (2). However, the level of expression of SREHP-MBP was low, and oral immunization with the *S. typhimurium*-SREHP-MBP strain did not induce mucosal or systemic antiamebic antibodies in mice or gerbils. In this study, we have utilized a new construct to achieve high-level expression of SREHP-MBP in an attenuated strain of *S. typhimurium*. More importantly, oral vaccination with the *S. typhimurium* expressing SREHP-MBP induces both mucosal and serum antiamebic antibodies in mice and gerbils, and oral vaccination prevents amebic liver abscess in gerbils after direct hepatic challenge with virulent amebae.

MATERIALS AND METHODS

Animals and amebae. Female gerbils (41 to 50 g) were obtained from Harlan Sprague Dawley (Indianapolis, Ind.). Female BALB/c mice (16 to 18 g) were obtained from Harlan Sprague Dawley. *E. histolytica* HM1:IMSS was grown on BYI-33 medium as previously described (6).

Bacterial strains. *S. typhimurium* χ4550 (Δ*cya* Δ*crp* Δ*asd*), which retains immunogenicity while being rendered avirulent by deletion of the adenyl cyclase (*cya*) and cyclic AMP receptor protein (*crp*) genes (10), was kindly provided by Roy Curtiss III, Washington University, St. Louis, Mo. *S. typhimurium* χ4550 was grown in Lennox medium supplemented with diaminopimelic acid (50 μg/ml; Sigma, St. Louis, Mo.) when Δ*asd* mutant strains without an *asd*-complementing plasmid were grown. Deletion of the *asd* gene allows transformation with the plasmid expression vector pYA3137 (provided by Curtiss), which contains a

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pUC18 origin of replication allowing a high copy number and the *asd* gene enabling selection for transformants without the use of antibiotic resistance. For the immunization experiments, overnight cultures were diluted 1/20 in Lennox medium and grown under aeration at 37°C to an optical density at 600 nm (OD_{600}) of 0.8 to 0.9 ($\sim 10^9$ CFU). Bacteria for oral gavage were quantified by plating serial dilutions on Lennox agar plates.

Construction of the SREHP-MBP expression vector. The plasmid pLi228 containing the *SREHP* gene and the *malE* gene encoding MBP has been previously described (2). The *malE-SREHP* fragment was cut from pLi228 by digesting with *Ssp*I and *Pst*I and then was ligated into pYA3137 which had been cut with *Sma*I and *Pst*I to create the pSS3137 plasmid. This plasmid was then used to transform *S. typhimurium* χ4550 grown in the absence of diaminopimelic acid. Control strains of χ4550 transformed with pYA3137 (Stm-Ctrl) were also prepared. Clones transformed with pSS3137 expressing the gene for the SREHP-MBP fusion protein (Stm-SREHP) were identified by restriction enzyme analysis and immunoblotting with the SREHP-specific monoclonal antibody 2D4 (8). Whole-cell lysates of the vaccine and control strains were also tested for lipopolysaccharide (LPS) expression by silver staining of lysates separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5).

Immunization and sampling. Immunization was performed as previously described (2). In brief, female BALB/c mice or gerbils were deprived of water and food for 4 h and then were given 50 μl of 10% sodium bicarbonate solution per orogastric gavage with a 21-gauge ball-tipped gavage needle. Five minutes later, they received by gavage 50 μl of 0.16 M phosphate-buffered saline (PBS), pH 7.0, containing 10^9 *S. typhimurium* cells harboring either pSS3137 (Stm-SREHP) or pYA3137 (Stm-Ctrl). Mice and gerbils were immunized on days 0, 7, and 21. Serum samples were obtained on days 0, 7, 21, and 28. Stool pellets were collected on days 0, 21, and 28 and were processed as previously described (21). Saliva samples were obtained from mice after subcutaneous injection of 0.11 ml of sterile pilocarpine nitrate (1 mg/ml; Sigma) on days 0 and 28. All samples were stored at −20°C for use in the enzyme-linked immunosorbent assay (ELISA).

ELISA and immunoblotting. To detect anti-SREHP immunoglobulin A (IgA) and IgG antibodies in stool, saliva, and serum specimens, 96-well ELISA plates were coated with *E. histolytica* HM1:IMSS trophozoites in PBS (10^4 per well) overnight at 4°C (21) or with recombinant SREHP-MBP at a concentration of 50 μg/ml. To measure anti-*Salmonella* LPS IgA and IgG antibodies in stool, saliva, and serum samples, 96-well ELISA plates were coated with a commercially available *S. typhimurium* LPS preparation (Sigma) in PBS at 100 ng per well overnight at 4°C. Plates were washed three times with PBS–0.05% Tween 20 and then blocked with 1% bovine serum albumin–PBS for 2 h at 37°C. Following two washes with PBS–0.05% Tween 20, duplicate samples of a 1:5 dilution of the stool samples, a 1:20 dilution of the saliva samples, and a 1:50 dilution of the serum samples were added to individual wells and incubated overnight at 4°C. Plates were washed as described above, and then biotin-labeled goat anti-mouse IgA and IgG (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) diluted 1:2,000 in PBS–0.05% Tween 20 were added to each well and incubated for 2 h at 37°C. We have determined that the anti-mouse IgA antibody also specifically binds gerbil IgA heavy chains (this was determined in part on the basis of the reactivity of this antibody with Jacalin-agarose [Pierce, Rockford, Ill.] purified gerbil IgA) (23). After three washes with PBS–0.05% Tween 20, a 1:4,000 dilution of horseradish peroxidase-streptavidin (Zymed) in PBS–0.05% Tween 20 was added to each well and incubated at room temperature for 1 h. Plates were washed as described above and then developed with a 1-mg/ml solution of 2,2-azino-*bis*(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) (Sigma). The plates were read in an ELISA reader at 405 nm.

Transformants of vaccine strain *S. typhimurium* χ4550 harboring either pSS3137 (Stm-SREHP) or pYA3137 (Stm-Ctrl) and colonies obtained from the spleens of mice immunized with Stm-SREHP or Stm-Ctrl were analyzed for expression of SREHP-MBP fusion protein by immunoblotting of SDS-PAGE-separated whole bacterial lysates with the murine monoclonal antibody 2D4 as previously described (8).

Hepatic challenge with amebae. On day 35 (14 days following the final booster immunization) control gerbils vaccinated with Stm-Ctrl and gerbils vaccinated with Stm-SREHP were challenged intrahepatically with 5×10^5 *E. histolytica* HM1:IMSS trophozoites by our previously described protocol (20). A matching group of nonvaccinated gerbils served as an additional control group. Seven days later, gerbils were sacrificed and amebic liver abscesses were assessed as previously described (20). The percent liver abscessed was calculated as the weight of the abscess divided by the liver weight before abscess removal.

RESULTS

High-level expression of SREHP-MBP can be achieved in *S. typhimurium* χ4550 transformed with pSS3137. Previously, we successfully expressed SREHP-MBP in *S. typhimurium*, using the plasmid pLi241 (2); however, expression could only be detected by immunoblotting with a monoclonal antibody to the SREHP molecule, and we could not detect SREHP-MBP by Coomassie staining of lysates from *S. typhimurium* transformed with pLi241 (2). To increase the level of SREHP-MBP expression, we utilized a new plasmid, pSS3137, derived from plasmid pYA3137, in which the p15A origin of replication used in pLi241 is replaced by a pUC18 origin of replication. As shown in Fig. 1A, this strategy was successful, as *S. typhimurium* χ4550 transformed with pSS3137 (Stm-SREHP) expressed high levels of SREHP-MBP (constituting 15 to 20% of the protein detectable on Coomassie-stained gels by densitometry). The identity of this protein as SREHP-MBP was confirmed by immunoblotting with monoclonal antibody 2D4 (Fig. 1B). Silver staining of SDS-PAGE-separated whole-cell lysates of Stm-SREHP and the Stm-Ctrl control strain demonstrated that each strain continued to express LPS as shown by the appropriate ladder pattern (data not shown) (5).

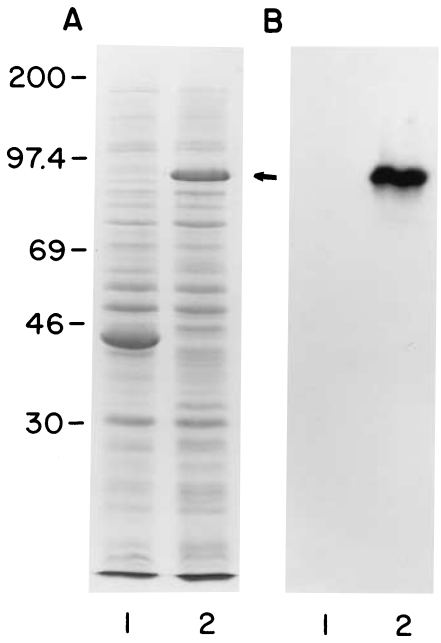

FIG. 1. Expression of SREHP-MBP in *S. typhimurium* χ4550 transformed with pSS3137 (Stm-SREHP). (A) Coomassie blue stained gels of SDS-PAGE-separated lysates from *S. typhimurium* χ4550 transformed with pYA3137 (Stm-Ctrl) (lane 1) and pSS3137 (Stm-SREHP) (lane 2). A species at 90 kDa (arrow), the predicted location of the SREHP-MBP fusion protein, is seen in Stm-SREHP lysates. (B) Immunoblots with monoclonal antibody 2D4, which recognizes the dodecapeptide repeat of SREHP, performed on SDS-PAGE-separated lysates of *S. typhimurium* χ4550 transformed with pYA3137 (Stm-Ctrl) (lane 1) and pSS3137 (Stm-SREHP) (lane 2). The 90-kDa SREHP-MBP protein is detected in lane 2.

Oral vaccination with Stm-SREHP induces mucosal and serum antiamebic antibodies in mice and gerbils. We were able to recover Stm-SREHP and Stm-Ctrl strains from the spleens of orally vaccinated mice at 7 days after the final vaccine dose. All Stm-SREHP strains recovered were expressing SREHP-MBP, as determined by immunoblot analysis (data not shown), and there was no significant difference in colony counts obtained from the spleens of five Stm-SREHP-vaccinated mice (mean ± standard error of the mean = 3,875 ± 1,200) and five Stm-Ctrl-vaccinated mice (mean ± standard error of the mean = 5,125 ± 1,100; $P > 0.15$). To study the immunogenicity of the Stm-SREHP strain, we orally vaccinated mice ($n = 10$ in each group) and gerbils ($n = 6$ in each of three separate trials) with Stm-SREHP or the control strain of *S. typhimurium* χ4550 transformed with pYA3137 (Stm-Ctrl). Since the SREHP molecule is primarily located on the surfaces of amebae (18) and the purpose of vaccination was to produce antiamebic antibodies, we have used an ELISA with

HM1:IMSS trophozoites as the target antigen to detect anti-SREHP–antiamebic antibody responses (21). We measured IgA and IgG anti-LPS and antiamebic antibodies in serum, stool, and saliva in mice and in serum and stool in gerbils. The murine response to vaccination is shown in Fig. 2. Mice vaccinated with Stm-SREHP produced IgA antibodies, in stool (Fig. 2A), saliva (Fig. 2B), and serum (Fig. 2C), that bound amebae. An IgG antiameba response was detected in serum samples from mice vaccinated with Stm-SREHP (Fig. 2C). In contrast, no antiamebic antibodies of either the IgA or IgG isotype were detected in stool, saliva, or serum samples from Stm-Ctrl-vaccinated mice. Similar results were seen when SREHP-MBP was used as the target antigen instead of amebic trophozoites (data not shown). After being vaccinated with either Stm-SREHP or Stm-Ctrl, mice produced detectable IgA anti-LPS antibodies in stool and saliva (Fig. 3A and B), and both IgA and IgG anti-LPS antibodies were detectable in serum at days 21 and 28 in Stm-SREHP- and Stm-Ctrl-vaccinated mice (Fig. 3C).

Vaccination of gerbils with Stm-SREHP induced IgA antiamebic antibodies which could be detected in stool (Fig. 4A) and IgG antiamebic antibodies which could be detected in serum (Fig. 4B). In contrast to the findings for mice, we could not detect IgA antiamebic antibodies in the serum of Stm-SREHP-vaccinated gerbils. Similar results were seen when SREHP-MBP was used as the target antigen (data not shown). Both Stm-SREHP and Stm-Ctrl vaccinations induced IgA anti-LPS antibodies which could be detected in the stool (Fig. 5A) and IgG anti-LPS antibodies which could be detected in the serum (Fig. 5B) of vaccinated gerbils.

Oral vaccination with Stm-SREHP protects gerbils from amebic liver abscess. Having established that oral vaccination with Stm-SREHP induces both mucosal and serum antiamebic antibody responses, we set out to determine whether the Stm-SREHP-vaccinated gerbils were protected against amebic liver abscess. Fourteen days following their final booster with Stm-SREHP or Stm-Ctrl and in three identical trials, groups of six gerbils and an additional nonvaccinated control group of five gerbils were challenged intrahepatically with virulent amebic trophozoites. There were two deaths before the 7-day end point in the Stm-Ctrl group and one death in the nonvaccinated group. Among the 18 gerbils receiving Stm-SREHP in the three trials, only 4 (22%) developed amebic liver abscesses (Table 1), while amebic liver abscesses developed in all 16 Stm-Ctrl-vaccinated gerbils and all 14 nonvaccinated control gerbils. In addition, the mean size of the abscesses in the four Stm-SREHP-vaccinated gerbils was significantly smaller than those detected in Stm-Ctrl-vaccinated and nonvaccinated gerbils.

DISCUSSION

The ultimate goal of these experiments is to develop an oral vaccine to prevent amebiasis. Attenuated salmonellae are attractive candidates to deliver *E. histolytica* antigens, because oral vaccination with salmonellae expressing heterologous antigens can induce mucosal IgA, serum IgG, and cell-mediated immune responses to the foreign antigens (4). This ability of salmonellae to stimulate multiple arms of the immune response may be especially important for a vaccine against amebiasis. Amebiasis begins as an intestinal infection, suggesting that mucosal IgA responses could be critical in preventing colonic colonization and disease. Support for this concept comes from an in vitro study showing that salivary secretory IgA from patients with previous *E. histolytica* infection can inhibit amebic adherence to mammalian cells (1). However,

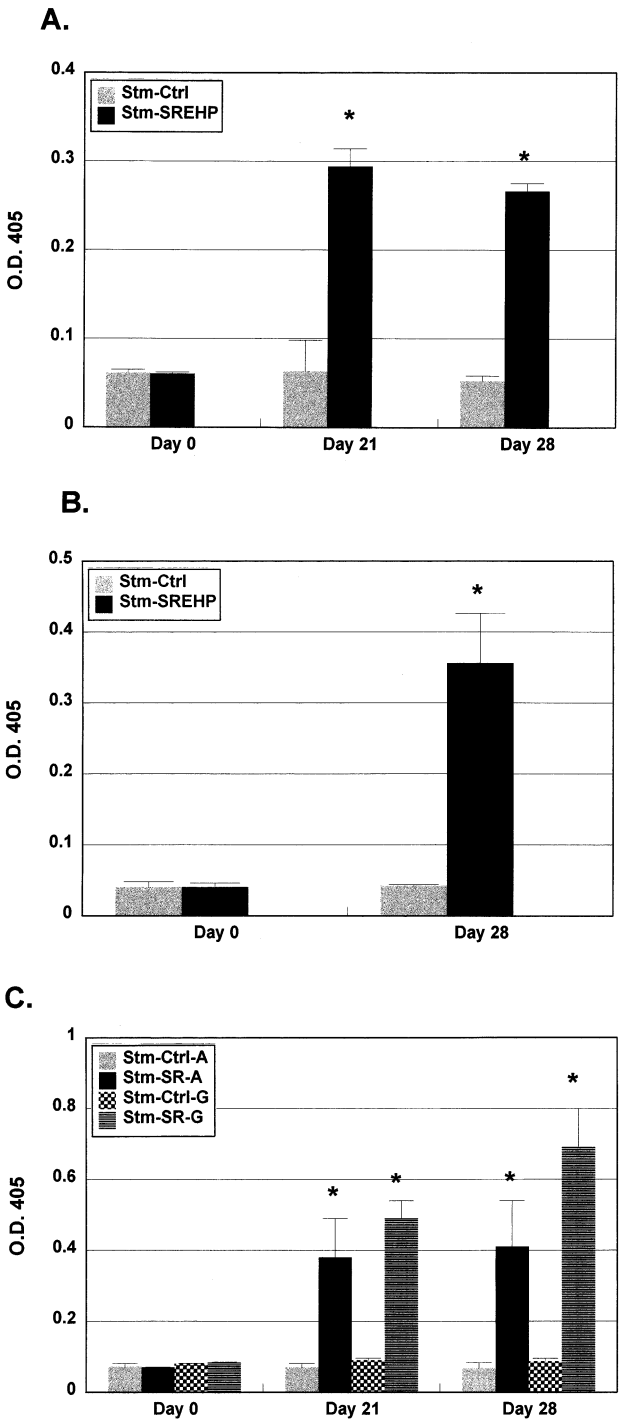

FIG. 2. Mice vaccinated with Stm-SREHP develop mucosal IgA antiamebic antibodies in stool and saliva and IgG and IgA antiamebic antibodies in serum. (A) IgA antiamebic response in stool samples from Stm-Ctrl- and Stm-SREHP-vaccinated mice before vaccination (day 0) and at days 21 and 28; (B) IgA antiamebic response in saliva obtained from Stm-Ctrl- and Stm-SREHP-vaccinated mice before vaccination (day 0) and at day 28; (C) IgA and IgG antiamebic response in serum from Stm-Ctrl- and Stm-SREHP-vaccinated mice before vaccination (day 0) and at days 21 and 28. Stm-SR-A refers to the IgA response in Stm-SREHP-immunized animals; Stm-SR-G refers to the IgG response in Stm-SREHP-immunized animals. Stm-Ctrl-A and Stm-Ctrl-G refer to the IgA and IgG responses, respectively, in Stm-Ctrl-vaccinated animals. Bars show mean values ± standard errors of the means (error bars) from groups of 10 mice. Asterisks indicate that the mean OD values of the indicated samples from the given days were significantly higher than those for the preimmune and control vaccinated animals ($P < 0.01$).

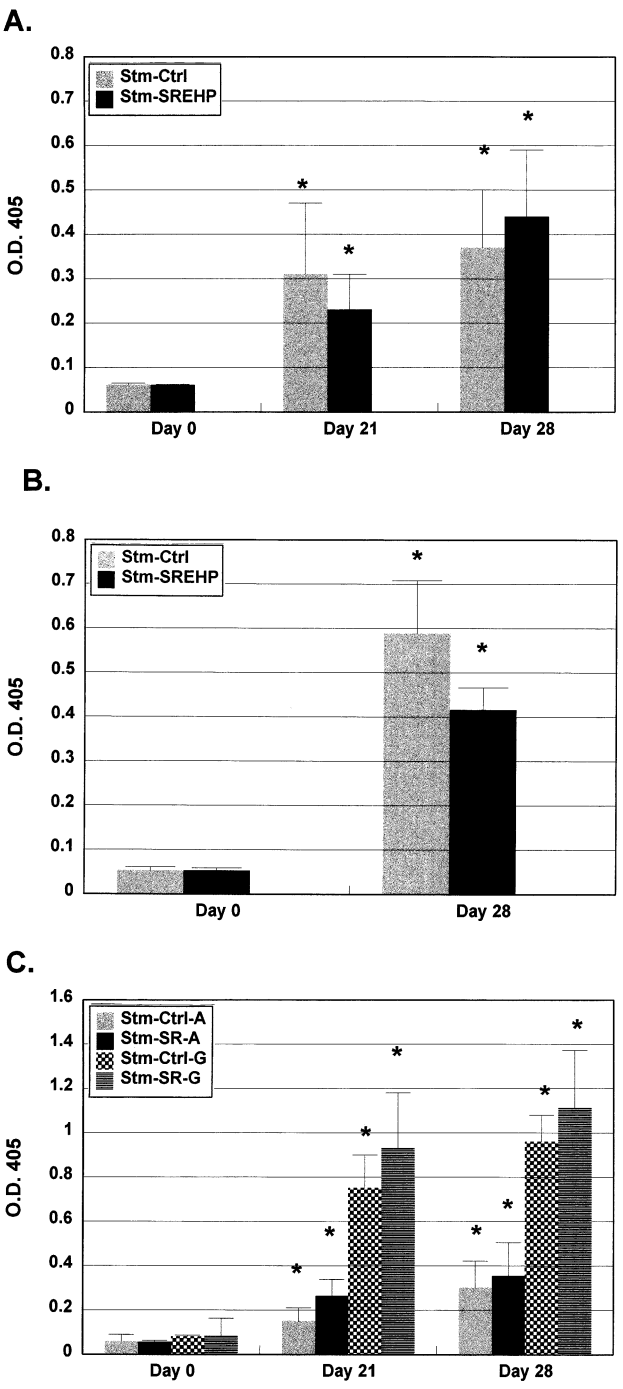

FIG. 3. Mice vaccinated with Stm-SREHP and Stm-Ctrl develop mucosal IgA anti-LPS antibodies in stool and saliva and IgG and IgA anti-LPS antibodies in serum. (A) IgA anti-LPS response in stool samples from Stm-Ctrl- and Stm-SREHP-vaccinated mice before vaccination (day 0) and at days 21 and 28; (B) IgA anti-LPS response in saliva obtained from Stm-Ctrl- and Stm-SREHP-vaccinated mice before vaccination (day 0) and at day 28; (C) IgA and IgG anti-LPS responses in serum from Stm-Ctrl- and Stm-SREHP-vaccinated mice before vaccination (day 0) and at days 21 and 28. Stm-SR-A refers to the IgA response in Stm-SREHP-immunized animals; Stm-SR-G refers to the IgG response in Stm-SREHP-immunized animals. Stm-Ctrl-A and Stm-Ctrl-G refer to the IgA and IgG responses, respectively, in Stm-Ctrl-vaccinated animals. Bars show mean values ± standard errors of the means (error bars) from groups of 10 mice. Asterisks indicate that the mean OD values of the indicated samples from the given days were significantly higher than those for the corresponding preimmune samples ($P < 0.01$).

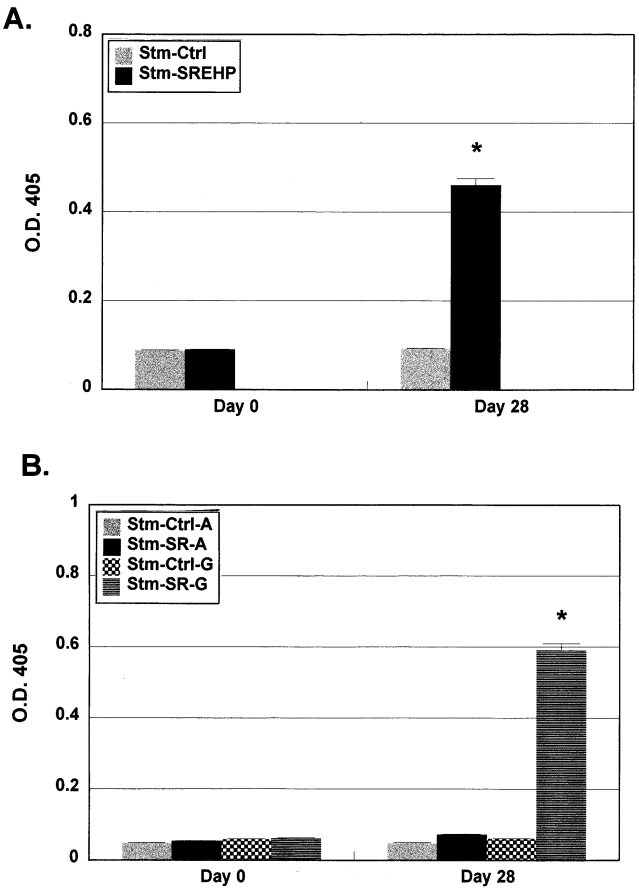

FIG. 4. Gerbils vaccinated with Stm-SREHP develop mucosal IgA antiamebic antibodies in stool and IgG antiamebic antibodies in serum. (A) IgA antiameba response in stool samples from Stm-Ctrl- and Stm-SREHP-vaccinated gerbils before vaccination (day 0) and at day 28; (B) IgA and IgG antiamebic responses in serum from Stm-Ctrl- and Stm-SREHP-vaccinated gerbils before vaccination (day 0) and at day 28. Stm-SR-A refers to the IgA response in Stm-SREHP-immunized animals; Stm-SR-G refers to the IgG response in Stm-SREHP-immunized animals. Stm-Ctrl-A and Stm-Ctrl-G refer to the IgA and IgG responses, respectively, in Stm-Ctrl-vaccinated animals. Bars show mean values ± standard errors of the means (error bars) from groups of 14 to 18 gerbils. Asterisks indicate that the mean OD values of the indicated samples from the given days were significantly higher than those for the preimmune and control vaccinated animals ($P < 0.01$).

the hallmark of amebic infection is *E. histolytica* invasion through colonic mucosae, with ulcer formation and penetration into submucosal tissues. Consistent with mucosal invasion, amebic dysentery is often associated with detectable serum IgG antiamebic antibodies, raising the possibility that the induction of serum IgG or cell-mediated immune responses could play some role in preventing or controlling intestinal disease. In addition, the most common extraintestinal manifestation of amebiasis, and a major cause of death associated with amebic infection, is amebic liver abscess, which results from the hematogenous spread of amebae from the colon to the liver. Protection from amebic liver abscess may require preexisting serum antiamebic antibodies (19), or antiamebic cell-mediated immune responses.

In this study we used attenuated salmonella to express a fusion protein of the amebic SREHP molecule and a bacterially derived MBP. The demonstrated immunogenicity of the hydrophilic SREHP molecule (15–17) and the proven protective efficacy of the recombinant SREHP-MBP vaccine made it

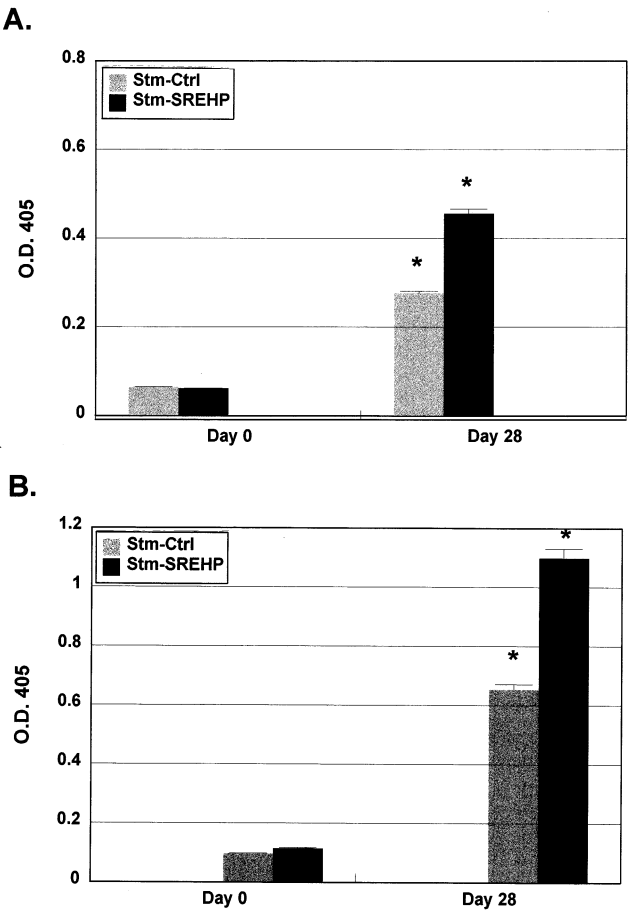

FIG. 5. Gerbils vaccinated with Stm-SREHP and Stm-Ctrl develop mucosal IgA anti-LPS antibodies in stool and IgG anti-LPS antibodies in serum. (A) IgA anti-LPS response in stool samples from Stm-Ctrl- and Stm-SREHP-vaccinated mice before vaccination (day 0) and at day 28; (B) IgG anti-LPS response in serum from Stm-Ctrl- and Stm-SREHP-vaccinated gerbils before vaccination (day 0) and at day 28. Bars show mean values ± standard errors of the means (error bars) from groups of 14 to 18 gerbils. Asterisks indicate that the mean OD values of the indicated samples from the given days were significantly higher than those for the corresponding preimmune samples ($P < 0.01$).

a reasonable candidate for these experiments. The SREHP-MBP fusion protein had been shown to be an effective parenteral vaccine against amebic liver abscess in the gerbil model of infection (20, 22), with vaccination inducing serum IgG antiamebic antibodies and delayed-type hypersensitivity responses to SREHP in immunized gerbils (20). The salmonella expression system used in these studies was designed by Curtiss and colleagues and employs a system of *trans* complementation of an *asd* gene deletion in *S. typhimurium* by the expression of the *asd* gene on the plasmid containing the foreign proteins to ensure stable expression and to allow selection of transformants without the use of genes encoding antibiotic resistance (9). Despite the known immunogenicity of SREHP-MBP and the demonstration of stable expression of the SREHP-MBP fusion protein in *S. typhimurium* recovered from vaccinated animals, our first attempt using *S. typhimurium* expressing SREHP-MBP to orally vaccinate mice and gerbils failed to induce mucosal or serum antiamebic antibodies in immunized animals (2). We hypothesized that this failure was secondary to inadequate levels of expression of the SREHP-MBP protein in the salmonellae. To increase the levels of SREHP-MBP expression in *S. typhimurium*, we constructed a new plasmid, pSS3137, derived from the plasmid pYA3137, which features a pUC origin of replication, allowing much higher plasmid copy numbers in transformed bacteria (10). *S. typhimurium* expressing pSS3137 (Stm-SREHP) produced significant quantities of SREHP-MBP and was capable of inducing mucosal IgA and serum IgG antiamebic antibodies in orally vaccinated mice and gerbils. These findings are consistent with the concept that the immune response to foreign proteins delivered in salmonella-based vaccines will depend to a large degree on both the level of expression of that antigen in salmonellae and the inherent immunogenicity of the foreign protein (10). Animals orally vaccinated with Stm-SREHP also produced mucosal and serum anti-LPS antibodies, with responses similar to those seen in animals vaccinated with the *S. typhimurium* control, suggesting that expression of SREHP-MBP did not diminish the underlying immunogenicity of the attenuated *S. typhimurium* strain. This is an important consideration in developing a dual vaccine that protects against both amebiasis and salmonella infection. While alterations in the drug administration schedule were not studied in these experiments, recent findings with a similar *S. typhimurium* strain expressing the hepatitis B core pre-S proteins demonstrated immunogenicity in mice after a single oral dose (10), raising the possibility that a single-dose regimen of the Stm-SREHP vaccine could be feasible.

A critical question addressed in these experiments was whether the antiamebic immune responses induced by Stm-SREHP vaccination can provide protection against invasive amebiasis. Because of the reproducibility of the results with the gerbil model of amebic liver abscess and the demonstrated ability of salmonellae to induce not only mucosal but also serum antibody and cell-mediated immune responses to foreign antigens, we elected to first study protection in the gerbil liver abscess model. We found levels of protection against amebic liver abscess in gerbils orally vaccinated with Stm-SREHP that approached or exceeded the levels seen with parenteral immunization with SREHP-MBP or other recombinant *E. histolytica* antigens (11, 12, 20, 22). This represents the first demonstration that an oral vaccine can successfully protect animals from amebic liver abscess, the major cause of mortality in human amebiasis. Studies examining the efficacy of Stm-SREHP vaccination in preventing intestinal amebiasis are an important next step, but such studies await the establishment of a reproducible animal model of intestinal infection.

Recently, we described an alternative approach to inducing mucosal immune responses to amebae, the oral vaccination of mice with a fusion protein containing the dodecapeptide repeat of SREHP fused to the B subunit of cholera toxin (CtxB–SREHP-12) (21). The CtxB–SREHP-12 fusion protein proved to be immunogenic, inducing mucosal IgA antiamebic responses in orally immunized mice. Studies using several different antigens, including tetanus toxoid, have suggested that

TABLE 1. Protection of gerbils from amebic liver abscess by oral vaccination

Vaccine group	No. of gerbils with liver abscess/no. of gerbils challenged (%)	% Liver abscessed (mean ± SD)
Stm-SREHP	4/18 (22)[a]	1.3 ± 0.1[b]
Stm-Ctrl	16/16 (100)	11.5 ± 7.5
Control	14/14 (100)	12.3 ± 8.1

[a] Significantly different from the corresponding values for Stm-Ctrl and control groups ($P < 0.001$).

[b] Significantly different from the corresponding values for Stm-Ctrl and control groups ($P < 0.01$).

immunization with foreign antigens and cholera toxin may stimulate a Th-2 dominated response to the antigen (with relatively higher levels of mucosal IgA), while foreign antigens expressed in salmonellae may stimulate a Th-1 dominated response (with stimulation of cell-mediated immune responses to the antigen) (for a review, see reference 13). In this regard, there did not appear to be a qualitative difference in mucosal IgA responses between CtxB–SREHP-12- and Stm-SREHP-vaccinated mice, but the titers of IgG antiamebic antibodies in serum appeared to be significantly higher for Stm-SREHP-vaccinated mice (23). Studies are now in progress to look at cell-mediated immune responses to amebae and SREHP in both Stm-SREHP- and CtxB–SREHP-12-vaccinated animals and to determine the duration of protective immunity provided by oral SREHP vaccination.

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