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Protection of Gerbils from Amebic Liver Abscess by Immunization with a Recombinant *Entamoeba histolytica* Antigen

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Amebiasis, infection by the intestinal protozoan parasite *Entamoeba histolytica*, is a leading parasitic cause of death. As a step in the development of a recombinant antigen vaccine to prevent *E. histolytica* infection, we looked at the ability of a recombinant version of the serine-rich *E. histolytica* protein (SREHP) to elicit a protective immune response against invasive amebic disease. Gerbils, a standard model for amebic liver abscess, were immunized with either a recombinant SREHP/maltose-binding protein (MBP) fusion, recombinant MBP alone, or phosphate-buffered saline (PBS), all combined with complete Freund's adjuvant. In the first trial (group 1), gerbils received a primary and two booster immunizations intraperitoneally; in the second trial (group 2), gerbils were immunized by a single intradermal injection. SREHP/MBP-immunized gerbils in both groups produced antibody to native SREHP and developed delayed-type hypersensitivity responses to recombinant SREHP. All gerbils were challenged by an intrahepatic injection with 5×10^4 virulent *E. histolytica* HM1:IMSS trophozoites. Complete protection from amebic liver abscess was seen in 64% of the SREHP/MBP-immunized gerbils in group 1 and in 100% of the SREHP/MBP-immunized gerbils in group 2. There was no protection observed in MBP- or PBS-immunized gerbils in either group. Our results indicate that the SREHP molecule has potential as a vaccine to prevent amebic infection and demonstrate that successful vaccination of animals with recombinant *E. histolytica* antigen vaccines is possible.

The intestinal protozoan parasite *Entamoeba histolytica* is a major cause of morbidity and mortality worldwide. Although effective antiamebic chemotherapy is available, *E. histolytica* still causes approximately 50,000 deaths yearly (20). Most of those deaths are secondary to the major extraintestinal complication of amebiasis, amebic liver abscess. Studies in humans and in animal models suggest that inducing protective immunity to amebic infection may be a feasible goal. Epidemiologic studies have shown that patients who are cured of amebic liver abscess are less likely to develop a subsequent infection than members of the general population (3, 14). Vaccination studies, using animal models of amebic liver abscess, have shown that animals immunized with crude preparations of *E. histolytica* lysates or intact *E. histolytica* trophozoites are protected against the development of amebic liver abscess after direct hepatic inoculation of amebic trophozoites (5–7, 9, 12, 15, 19). A single study has reported protection in an animal model of amebiasis obtained by vaccination with a defined amebic antigen (13). A significant limitation of vaccine studies using *E. histolytica*-derived antigens is the ability to obtain the quantities of amebic proteins necessary for large-scale immunization.

To approach this problem, we have produced a recombinant version of the serine-rich *E. histolytica* protein (SREHP), a highly immunogenic amebic surface glycoprotein that possesses multiple hydrophilic dodecapeptide and octapeptide repeats (16). Most patients with amebic liver abscess produce antibodies to SREHP (18), and polyclonal antiserum to a recombinant SREHP molecule inhibited amebic adhesion to mammalian cells (16). The SREHP molecule has been expressed as both TrpE and maltose-binding protein (MBP) fusion proteins (11, 16). We immunized gerbils, a standard model of amebic liver abscess (2), with recombinant SREHP/MBP or with the MBP alone and examined the resistance of immunized and control animals to direct hepatic inoculation with virulent amebae. Herein we report that immunization with recombinant SREHP can protect gerbils from amebic liver abscess.

MATERIALS AND METHODS

Gerbils. Adult (6- to 8-week-old) female gerbils were obtained from Harlan Sprague Dawley (Indianapolis, Ind.).

Amebae. A hamster liver-passaged strain of *E. histolytica* HM1:IMSS (originally provided by V. Tsutsumi, Center for Research and Advanced Studies, National Polytechnical Institute, Mexico City, Mexico) was grown in BI-S-33 medium (4) and was passaged monthly through gerbil livers to ensure continued virulence (1).

Expression and purification of recombinant SREHP/MBP. Expression and purification of the recombinant SREHP/MBP fusion protein by using the pMAL system (New England Biolabs, Beverly, Mass.) has been described in detail elsewhere (11). The SREHP/MBP fusion protein contained amino acids 10 through 222 of the derived SREHP sequence (16) (thus eliminating the putative signal sequence and transmembrane region) fused to the 42-kDa MBP molecule. The isopropyl-β-D-thiogalactoside (IPTG)-induced SREHP/MBP fusion protein was purified from the supernatant of *Escherichia coli* lysates by affinity chromatography using monoclonal antibody 2D4, which recognizes the dodecapeptide repeat of the SREHP molecule (11). The purity of the SREHP/MBP fusion

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protein preparation was assessed by Coomassie staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-separated proteins (11). Expression of MBP alone was also performed by using the pMAL vector, and the MBP was purified from *E. coli* lysates by using an amylose column as specified by the manufacturer (New England Biolabs). Purity of the MBP preparation was assessed by Coomassie staining of SDS-PAGE-separated proteins.

For some studies, the SREHP/MBP fusion protein was cleaved into the SREHP and MBP peptides by using factor Xa protease (0.0125 U/μl) as specified by the manufacturer (New England Biolabs). SREHP and MBP were then separated by fast protein liquid chromatography (FPLC), using a Superose gel filtration column (Pharmacia LKB Biotechnology, Piscataway, N.J.) as previously described (11). This procedure allows complete separation of recombinant SREHP from the MBP fusion partner (11).

Immunization of gerbils with SREHP/MBP and MBP. In the first trial (group 1), adult female gerbils were immunized with 100 μg of SREHP/MBP (n = 14) or MBP (n = 10) in a volume of 75 μl with an equal volume of complete Freund's adjuvant (CFA) intraperitoneally. An additional control group of 11 gerbils received phosphate-buffered saline (PBS) and CFA. On days 28 and 47, gerbils received a booster immunization with 100 μg of fusion protein or PBS in incomplete Freund's adjuvant. On day 68, gerbils were challenged with amebae as described below.

In the second trial (group 2), adult female gerbils received a single intradermal immunization with 150 μg of SREHP/MBP (n = 9) or 150 μg of MBP (n = 9) in a volume of 75 μl combined with an equal volume of CFA. An additional five gerbils received a single intradermal injection with 75 μl of PBS combined with 75 μl of CFA. Intradermal immunization was performed under ketamine and xylazine anesthesia. A 2-cm^2 region of abdominal skin was shaved and scrubbed with povidone-iodine, and then the vaccine preparation was injected intradermally to raise a single visible bleb, using a 26-gauge needle and 1-ml syringe.

Western blotting (immunoblotting) and ELISA. Serum was obtained from all gerbils by cardiac puncture (under ketamine and xylazine anesthesia) immediately prior to primary vaccination (preimmune serum) and on days 42 and 61 in group 1 and on day 35 in group 2. Reactivity of individual immune and preimmune gerbil serum, diluted 1:500, 1:1,000, 1:2,500, and 1:5,000, against SDS-PAGE-separated *E. histolytica* HM1:IMSS trophozoite lysates was determined by Western blotting using rabbit anti-hamster immunoglobulin G (Sigma Chemical, St. Louis, Mo.) as a secondary antibody and ^{125}I-labeled staphylococcal protein A (16). Serum from individual gerbils immunized with the recombinant MBP was examined for reactivity against the immunizing antigen in SDS-PAGE-separated *E. coli* lysates from pMAL-expressing bacteria. The serum obtained from SREHP/MBP-immunized gerbils in group 1 from day 61 and from SREHP/MBP-immunized gerbils in group 2 from day 35 was also analyzed by enzyme-linked immunosorbent assay (ELISA) for reactivity with *E. histolytica* HM1:IMSS trophozoite lysates (10 μg/ml), using our standard protocol (17). Alkaline phosphatase-conjugated goat anti-hamster immunoglobulin G (Sigma) was used as the secondary antibody. Serum dilutions from 1:500 to 1:20,000 were examined, and the highest dilution which gave an optical density of ≥0.200 at 405 nm was recorded.

Measurement of delayed-type hypersensitivity (DTH). On day 66 (under ketamine and xylazine anesthesia), group 1 gerbils had their footpad thickness measured with calipers and then received 10 μg of either purified recombinant SREHP or MBP, in a volume of 25 μl, injected into the left footpad and 25 μl of sterile saline injected into the right footpad. Footpad thickness was measured by calipers 48 h later. The same protocol was used for group 2 gerbils starting on day 42.

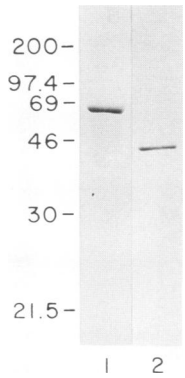

FIG. 1. SDS-PAGE analysis of recombinant vaccines used in this study. Lane 1, 25 μg SREHP/MBP fusion protein; lane 2, 25 μg of MBP. The approximate sizes in kilodaltons of molecular size standards are shown at the left.

Hepatic inoculation of amebae. On day 68 of the protocol for group 1 and on day 47 for group 2, gerbils were anesthetized with ketamine and xylazine. After a povidone-iodine scrub, a vertical incision, 1.5 cm in length, was made in the anterior abdominal wall and peritoneum, and the liver was visualized. One hundred microliters of amebic inoculum containing 50,000 *E. histolytica* HM1:IMSS trophozoites was injected from a 1-ml tuberculin syringe via a 26-gauge needle into the liver such that a visible bleb was raised on the liver surface (10). The peritoneum was closed with 4-0 chromic sutures, and the abdominal wall was closed with 7-mm Michel clips. Seven days later, animals were sacrificed; the entire liver was removed and weighed, and any abscess seen grossly was resected and weighed. The percent liver abscessed was calculated as the weight of the abscess divided by the liver weight before abscess removal. A small specimen from each abscess was cultured in BI-S-33 medium (4). Liver abscesses, as well as visually normal livers, were fixed in formalin, sectioned, and stained with hematoxylin and eosin for histological examination. Liver abscesses in the gerbils were diagnosed on the basis of the gross appearance, and the diagnosis was confirmed by the finding of a positive culture for amebae and compatible histologic findings (amebic trophozoites visible in the abscess tissue).

Statistical analysis. The comparison of vaccine efficacy was performed by using Fisher's exact test (two-tail values presented). Student's t test was used to assess the significance of differences between means in measurements of liver abscess size, DTH responses, and serologic assays.

RESULTS

Immunogenicity of the SREHP/MBP recombinant protein. Before immunization of gerbils, the purity of the recombinant SREHP/MBP fusion protein preparation and the purity of recombinant MBP antigen preparation were assessed by SDS-PAGE. As shown in Fig. 1 (lane 1), the 68-kDa SREHP/MBP fusion protein was the predominant band seen after 2D4 affinity chromatography purification of lysates from *E. coli* expressing SREHP/MBP. The 42-kDa MBP (Fig. 1, lane 2) was the predominant band seen after amylose affinity chroma-

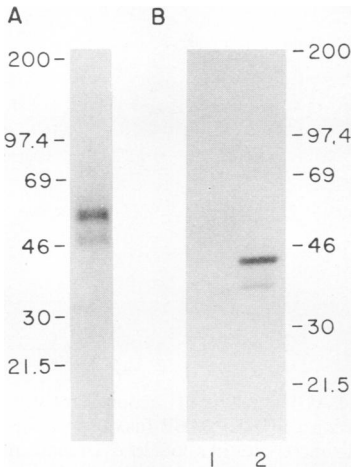

FIG. 2. Gerbils immunized with recombinant SREHP/MBP develop antibody responses to the native SREHP molecule. (A) Immunoblot of day 61 serum (1:5,000 dilution) from a group 1 gerbil immunized with SREHP/MBP reacted with HM1:IMSS trophozoite lysates. (B) Immunoblot of day 61 serum (1:5,000 dilution) from a group 1 gerbil immunized with recombinant MBP reacted with HM1:IMSS trophozoite lysates (lane 1) and bacterial lysates from *E. coli* expressing the pMAL plasmid (lane 2). The approximate sizes in kilodaltons of molecular size standards are shown to the side of each panel.

tography purification of lysates from pMAL-expressing *E. coli*. Antibody responses to amebic lysates following immunization with recombinant SREHP/MBP, recombinant MBP, or PBS were assessed in all gerbils. By day 61 (14 days following the second booster immunization), all gerbils immunized with SREHP/MBP fusion protein in group 1 had antibody titers of 1:5,000 against the native SREHP molecule, as measured by Western blotting. A sample Western blot of immune serum obtained at day 61 from an SREHP/MBP-immunized gerbil and reacted with *E. histolytica* HM1:IMSS lysates is shown in Fig. 2A. Reactivity with the 47/52-kDa native SREHP molecule is seen (16). All gerbils immunized with MBP alone showed reactivity against the recombinant 42-kDa MBP at a serum dilution of 1:5,000 at days 42 and 61 (Fig. 2B, lane 2). Sera from MBP-immunized (Fig. 2B, lane 1) and PBS-immunized (data not shown) gerbils did not react with amebic lysates at serum dilutions of 1:500 in Western blots. The magnitude of the anti-native SREHP antibody titers in SREHP/MBP-immunized gerbils (at day 61) was established by ELISA. The geometric mean of the maximum reciprocal titer for the 14 gerbils in group 1 was 11,096 ± 1,899 (range, 7,500 to 15,000). Preimmune serum and serum from PBS- and MBP-immunized gerbils did not react with amebic lysates in the ELISA at a dilution of 1:500.

Among SREHP/MBP-immunized gerbils in group 2, in serum obtained at day 35, three of nine had antibody titers of 1:1,000, and the remaining six gerbils had antibody titers of 1:2,500 against the native SREHP molecule, as measured by Western blotting (data not shown). All MBP-immunized gerbils had antibody titers of 1:2,500 against the recombinant MBP molecule in day 35 sera tested by Western blotting (data not shown). Neither MBP- nor PBS-immunized gerbils showed reactivity with amebic lysates at serum dilutions of 1:500 in Western blots. The magnitude of the anti-amebic lysate antibodies in the nine SREHP/MBP-immunized gerbils in group 2 at day 35 (12 days prior to amebic challenge), as measured by ELISA, was significantly lower than that seen 7 days prior to amebic challenge in the SREHP/MBP-immunized gerbils in group 1 (geometric mean of the maximum reciprocal titer, 4,354 ± 3,065; range, 2,500 to 10,000; $P \leq 0.001$ for the difference between SREHP/MBP-immunized gerbils in group 1 and group 2).

TABLE 1. Gerbils immunized with recombinant SREHP/MBP develop DTH responses to recombinant SREHP

Animals (*n*)	Footpad swelling (mm)[a] with:	
	SREHP challenge	MBP challenge
Group 1 gerbils vaccinated with:		
SREHP/MBP (6)	1.9 ± 1.1[b]	
PBS (6)	0.2 ± 0.1	
MBP (10)	0.1 ± 0.1	
Group 2 gerbils vaccinated with:		
SREHP/MBP (9)	2.6 ± 0.9[c]	
PBS (5)	0.1 ± 0.1	
MBP (9)		1.8 ± 0.5

[a] Mean ± standard deviation of the differences between the width of the left footpad (SREHP or MBP challenged) and the width of the right footpad (saline challenged) for gerbils in each group.

[b] $P \leq 0.001$ for the difference in footpad swelling in response to SREHP between SREHP/MBP-immunized gerbils and the PBS- or MBP-immunized gerbils in each group.

[c] $P \leq 0.001$ for the difference in footpad swelling in response to SREHP between SREHP/MBP- and PBS-immunized gerbils in group 2.

DTH responses to recombinant SREHP. To determine whether immunization with recombinant SREHP/MBP induced cell-mediated immune responses to SREHP, we measured the DTH responses to purified SREHP (cleaved and separated from the MBP fusion partner) in 6 of the 14 SREHP/MBP-immunized gerbils, 6 of the PBS-immunized gerbils, and all 10 MBP-immunized gerbils in group 1. DTH responses to purified cleaved recombinant SREHP were measured in all nine SREHP/MBP-immunized gerbils and all five PBS-immunized gerbils in group 2, while DTH responses to purified MBP were measured in the nine MBP-immunized gerbils in group 2. As summarized in Table 1, the six SREHP/MBP-immunized gerbils in group 1 showed a DTH response to SREHP. A similar DTH response to recombinant SREHP was seen in the nine SREHP/MBP-immunized gerbils in group 2. In contrast, no difference in the thickness of SREHP and saline inoculated footpads at 48 h was seen in the PBS-immunized gerbils in either group 1 or group 2. The 10 MBP-immunized gerbils in group 1 did not develop a DTH response to recombinant SREHP. The nine MBP-immunized gerbils in group 2 showed significant DTH responses to MBP.

Protection experiments. As summarized in Table 2, gerbils immunized with recombinant SREHP/MBP were protected against amebic liver abscess. Of the 14 gerbils immunized with the recombinant SREHP/MBP antigen in group 1, 9 of 14 (64%) failed to develop an amebic abscess after direct hepatic inoculation with virulent amebae. In contrast, all 11 PBS-immunized animals and all 10 gerbils immunized with the recombinant MBP alone in group 1 developed amebic liver abscesses after intrahepatic challenge with virulent amebae. In group 2, all nine gerbils immunized intradermally with SREHP/MBP were protected against amebic liver abscess, while the nine gerbils receiving intradermal immunization with recombinant MBP and the five gerbils immunized with PBS all developed amebic liver abscess. While all gerbils receiving intradermal immunization with recombinant SREHP/MBP were protected from amebic liver abscess, the difference in protective efficacy between intraperitoneal immunization with SREHP/MBP (group 1) and intradermal immunization (group

TABLE 2. Gerbils immunized with recombinant SREHP are protected against amebic liver abscess

Immunogen	No. with amebic liver abscess/no. challenged (%)	% Liver abscessed ± SD
Group 1, intraperitoneal		
PBS	11/11 (100)	9.6 ± 8.5
MBP	10/10 (100)	20.0 ± 7.4[a]
SREHP/MBP	5/14 (36)[b]	12.9 ± 7.3
Group 2, intradermal		
PBS	5/5 (100)	10.1 ± 6.3
MBP	9/9 (100)	18.4 ± 9.3[c]
SREHP/MBP	0/9 (0)[d]	

[a] $P \leq 0.01$ for the difference between the liver abscess size in MBP- and PBS-immunized animals; $P \geq 0.09$ for the difference between the liver abscess size in MBP- and SREHP/MBP-immunized animals in group 1; $P \geq 0.5$ for the difference in liver abscess size between PBS- and SREHP/MBP-immunized gerbils in group 1.

[b] $P = 0.001$ for the difference in developing liver abscesses between SREHP/MBP- and PBS-immunized gerbils in group 1; $P = 0.002$ for the difference in developing liver abscesses between SREHP/MBP- and MBP-immunized animals in group 1.

[c] $P \geq 0.1$ for the difference in abscess size between MBP- and PBS-immunized gerbils in group 2.

[d] $P \leq 0.001$ for the difference in developing liver abscess between SREHP/MBP- and PBS- or MBP-immunized gerbils in group 2.

2) was not statistically significant ($P \geq 0.11$). Examination of liver abscess size revealed no significant differences in the percentage of the liver abscessed between the five vaccine failures in the SREHP/MBP group compared with the PBS control group. However, gerbils immunized with the recombinant MBP alone had significantly larger abscesses than the PBS controls in group 1, and a similar trend (which did not reach statistical significance) was noted in group 2.

To determine whether protection from amebic liver abscess in group 1 could be correlated with the level of anti-SREHP antibody titers present in immunized gerbils before intrahepatic challenge with amebae, we compared the maximum anti-native SREHP titer at day 61 (prior to challenge) for gerbils protected in group 1 and for the five group 1 SREHP/MBP vaccine failures. There was no quantitative difference in the antibody response to native SREHP between the gerbils protected in group 1 and the group 1 SREHP/MBP vaccine failures (geometric means of reciprocal titers, 10,914 ± 3,121 versus 11,429 ± 1,977; ($P \geq 0.3$). Among the six gerbils in group 1 for which DTH responses to recombinant SREHP were measured, the magnitude of the prechallenge DTH response to recombinant SREHP in the two SREHP/MBP-immunized gerbils that developed amebic liver abscess (footpad swelling of 1.9 ± 0.6 mm) was not different from the DTH responses seen in the protected gerbils (footpad swelling of 1.9 ± 1.4 mm; $P = 0.98$).

DISCUSSION

This study was designed to determine whether immunization with a recombinant version of SREHP could prevent amebic liver abscess in gerbils, a standard model for hepatic amebiasis. The native SREHP molecule is a major surface antigen of *E. histolytica* and has been implicated in amebic adhesion to target cells (16). SREHP is a very hydrophilic protein and contains multiple dodecapeptide and octapeptide repeats, with a secondary structure that bears some resemblance to that of the circumsporozoite proteins of malaria (16). Previous studies have suggested that at least some of the epitopes of the native SREHP molecule are highly conserved among pathogenic *E. histolytica* strains. More than 80% of patients with invasive amebiasis develop antibodies to recombinant SREHP (18), and a recent report suggested that the nucleotides encoding the repeating units of SREHP may be highly conserved among both pathogenic and nonpathogenic *E. histolytica* strains (8). We had previously shown that a recombinant SREHP/TrpE fusion protein is immunogenic in rabbits (16). In this study, we used two forms of immunization: multiple intraperitoneal immunizations with recombinant SREHP/MBP or a single depot intradermal immunization with recombinant SREHP/MBP. Both techniques have been successful in previous vaccine studies using native amebic antigens or immunization with amebic trophozoites (5, 7, 13). We found that the intraperitoneally immunized gerbils (group 1) developed significant antibody titers against the native SREHP molecule following primary immunization and two booster immunizations with the recombinant SREHP/MBP protein. In addition, multiple immunizations with recombinant SREHP/MBP primed gerbils for a DTH response to a purified SREHP molecule which had been chemically cleaved from the MBP fusion partner. A single depot intradermal immunization with recombinant SREHP/MBP also induced antibody to native SREHP and DTH responses to cleaved recombinant SREHP (group 2). We did not examine whether immunization with recombinant SREHP/MBP induced DTH responses to native amebic proteins in this study because of concerns that the administration of even small quantities of native amebic proteins to the gerbils before intrahepatic challenge with amebae might obscure the effect of the recombinant vaccine.

Immunization of gerbils with the recombinant SREHP molecule completely protected 64% of animals from amebic liver abscess in group 1 and 100% of the SREHP/MBP-immunized animals in group 2. The difference in protective efficacy of SREHP/MBP immunization between groups 1 and 2 was not statistically significant ($P \geq 0.11$). A second trial of intraperitoneal immunization of gerbils with recombinant SREHP/MBP, performed as part of a study to compare the efficacies of different recombinant *E. histolytica* antigens in protection against amebic liver abscess, has given similar results (22). The 64% level of vaccine efficacy seen in the group 1 immunized animals in this trial is somewhat lower than that seen in studies using other animal models, in which animals were immunized with crude amebic lysates or fractionated amebic proteins (6, 7, 9, 12, 15, 19). However, the degree of protection seen with intraperitoneal immunization with recombinant SREHP is similar to that seen in the only previous *E. histolytica* vaccine study using a single defined amebic antigen, where a calculated vaccine efficacy of 67% for gerbils immunized with the native 220-kDa surface adhesin of *E. histolytica* was reported (13). The protection seen after immunization with recombinant SREHP appeared to be of an all-or-nothing nature. The group 1 gerbils that developed liver abscess despite SREHP/MBP immunization had liver abscesses equivalent in size to those seen in the PBS control group. This result differs from that reported for immunization with the native 220-kDa molecule; in that case, gerbils which developed liver abscesses after 220-kDa antigen immunization had abscesses significantly larger than those in gerbils receiving sham immunizations, suggesting possible immunosuppressive effects of 220-kDa antigen immunization in some animals (13). While no exacerbation of disease was recognized in gerbils immunized with SREHP/MBP, we found that group 1 gerbils intraperitoneally immunized with MBP alone had liver abscesses significantly larger than those of gerbils in the PBS control group. The reason for larger abscesses in the MBP-vaccinated gerbils is unknown, but this finding raises questions about whether

SREHP/MBP represents the optimal fusion protein for a recombinant SREHP vaccine.

How immunization with recombinant SREHP protected gerbils from amebic liver abscess in this model was not directly addressed in this study. We have recently found that passive immunization with heterologous antibody to recombinant SREHP can protect mice with severe combined immunodeficiency from amebic liver abscess, suggesting that antibody could be mediating protection in the gerbil model (21). However, a comparison of the anti-native SREHP antibody titers in group 1 SREHP/MBP-immunized gerbils before intrahepatic challenge with amebae did not reveal a significant difference between gerbils that were protected from amebic liver abscess and those that developed disease. In addition, the gerbils immunized intradermally with recombinant SREHP/MBP (group 2) had anti-native SREHP antibody titers before amebic challenge that were significantly lower than those of the group 1 gerbils but had a similar level of protection. The level of antiamebic antibody titers after immunization has not correlated with protective immunity in two other *E. histolytica* vaccine studies (6, 13). Additional studies will be necessary to determine whether antibodies play a role in the protection seen with SREHP/MBP-immunized gerbils and, if so, whether efficacy might correlate with a particular property of the antibodies, such as anti-amebic adherence activity, rather than the absolute titer against amebic lysates. With the identification of SREHP as a protective antigen, further studies of recombinant SREHP-induced cell-mediated immune responses to native SREHP may provide information on the role of cell-mediated immunity in protection against amebic liver abscess.

In summary, we have shown that immunization with a recombinant version of the amebic SREHP molecule can protect against amebic liver abscess in the gerbil model. This finding clearly establishes the SREHP antigen as a potential vaccine candidate to prevent amebiasis and demonstrates that successful vaccination of animals with recombinant *E. histolytica* antigens is feasible.

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