91% in Mexico City (88). Differences in the observed rates of anti-SREHP antibody production may have been due to differences in the timing of serum sampling during the course of acute illness. They also raise the possibility that local populations have differing abilities to produce anti-SREHP antibodies or that differences in the number of SREHP repeats in different *E. histolytica* isolates affect immunogenicity (88).

Zhang et al. tested the ability of recombinant SREHP to elicit a protective immune response against amoebic liver abscess in gerbils. Gerbils were immunized either subcutaneously in a single shot or intraperitoneally in a series of three shots with a recombinant SREHP/maltose-binding protein (MBP) fusion protein combined with complete Freund's adjuvant. Immunization completely prevented amoebic liver abscess following intrahepatic challenge in 64% of gerbils immunized intraperitoneally and 100% of gerbils immunized with a single subcutaneous shot. All of the immunized animals developed delayed-type hypersensitivity reactions (89). African green monkeys immunized with three doses of the SREHP/MBP fusion protein developed serum antiamoebic antibodies ten days after the first booster. Unfortunately, the control monkeys in this trial did not develop liver abscesses following intrahepatic challenge, so vaccine efficacy could not be assessed (90).

Screening of cDNA libraries also identified the 29-kDa cysteine–rich *E. histolytica* antigen, another immunogenic protein that may be suitable for inclusion in a vaccine (80). The 29-kDa antigen appears to be a thiol-dependent peroxidase, since it possesses hydrogen peroxide removing capacity in the presence of reducing agents such as thioredoxin (94,95). It may, therefore, protect *E. histolytica* from oxidative attack by activated neutrophils and macrophages. The location of the 29-kDa antigen within the amoeba remains controversial. Immunofluorescent staining of formalin-fixed cells with monoclonal antibodies shows the protein within both the nucleus and cytoplasm (94,95). Intraperitoneal immunization of gerbils with a recombinant fusion protein based on the 29-kDa protein and Titermax adjuvant elicited production of antigen-specific IgG and was partially protective (54% vaccine efficacy) against amoebic liver abscess following intrahepatic challenge with virulent trophozoites (96).

The cysteine proteinases and the amoebapore are additional amoebic proteins associated with virulence that must be considered. Each has yet to be evaluated as a potential vaccine component. Numerous studies document the central role of amoebic cysteine proteinases in penetration of host tissues and in evasion of host defenses via degradation of IgA, IgG, C3a, and C5a. Patients with amoebic liver abscess, moreover, develop antibodies to histolysain (*EhCP2*), and the use of protease inhibitors in SCID mice reduces the size of liver abscesses following intrahepatic injection of trophozoites. The recombinant amoebapore's cytotoxicity toward eukaryotic cells prohibits its use in a vaccine. Identification of antigenic regions within this peptide, however, should yield other possible vaccinogens.

## ORAL VACCINES

Two major oral vaccine strategies have been used: the incorporation of amoebic antigens into attenuated bacterial strains and the creation of fusion proteins composed of amoebic antigens and cholera toxin or its subunits. An effective oral vaccine against *E. histolytica* could have several advantages over parenteral preparations. Direct stimulation of the gut-associated lymphoid tissue (GALT) might stimulate production of secretory IgA more effectively than parenteral immunization, and prevent both colonization and invasive disease (97). By establishing a limited invasive infection in the host; moreover, an oral vaccine carried by an attenuated bacterial strain might provide more prolonged immunity than parenteral vaccines based on the same antigens. Combination vaccines providing protection against multiple organisms may also be possible. For example, immunization with attenuated *Salmonella typhi* strains engineered to express amoebic antigens might protect against both amebiasis and typhoid fever. Finally, the lower cost and ease of administering an oral vaccine would increase acceptance in developing nations.

An oral attenuated vaccine for typhoid fever is currently in use in humans. Foreign antigens expressed in attenuated *Salmonella* species can effectively stimulate both cell-mediated immunity and production of secretory IgA (98). Oral immunization of mice and gerbils with an attenuated strain of *Salmonella typhimurium* that expresses the SREHP/MBP fusion protein at high levels resulted in production of secretory IgA and serum IgG. Anti-lipopolysacharide (LPS) antibodies also developed in both sham immunized and immunized animals, suggesting that the amoebic antigen did not impair the immune response to the *Salmonella* infection. A vaccine protective against both, therefore, might be possible. Following intrahepatic injection with amoebic trophozoites, 100% of control gerbils and only 22% of immunized gerbils developed abscesses in this study (99). In another study, oral immunization of gerbils with *Salmonella dublin* expressing a fragment of the Gal/GalNac lectin resulted in significant reduction in mean abscess weight, but no significant difference in the number of animals developing abscesses. No serum antiamoebic antibody production was observed in this study, suggesting that the observed protection may have been cell mediated (98). The plasmid carrying the lectin fragment, however, was somewhat unstable in vitro; higher or more prolonged expression of the antigen may have resulted in antibody production and in greater vaccine efficacy (98).

Cholera toxin has two subunits, a 28-kDa A subunit with ADP-ribosylating activity, and an 11.5-kDa B or binding subunit. The A subunit contains $A_1$, the active toxin domain, and $A_2$, which noncovalently links subunit A to five B subunits. A pentamer of B subunits binds the intestinal epithelium. Whole cholera toxin stimulates production of serum IgG and secretory IgA when orally administered, and also stimulates immunity to coadministered antigens (100). In humans, the B subunit retains some of whole cholera toxin's oral adjuvant properties. Parenteral immunization of rats with native Gal/GalNac lectin in complete Freund's adjuvant followed by intra-Peyer's patch injection of lectin with cholera toxin's B subunit stimulates production of anti-lectin secretory IgA (101). Oral immunization of mice with the recombinant LC3 portion of the lectin and whole cholera toxin induced production of secretory IgA capable of inhibiting adherence of amoebic trophozoites to CHO cells. Interestingly, there was a negative correlation between intestinal IgA production and serum IgA and IgG titers in this study (102). High-dose oral immunization with streptococcal antigens by other investigators has resulted similarly in a strong mucosal immune response with no systemic antibody production, while lower doses led both to mucosal and systemic antibody production.

A potential limitation of strategies combining recombinant peptides with cholera toxin's B subunit is that large