Adherence-Inhibitory Intestinal Immunoglobulin A Antibody Response in Baboons Elicited by Use of a Synthetic Intranasal Lectin-Based Amebiasis Subunit Vaccine

Mohamed D. Abd Alla,1 Gary L. White,2 Tyson B. Rogers,1 Max E. Cary,2 David W. Carey,2 and Jonathan I. Ravdin1*

Department of Medicine, University of Minnesota, Minneapolis, Minnesota,1 and Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma2

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We designed an amebiasis subunit vaccine that is constructed by using four peptide epitopes of the galactose-inhibitable lectin heavy subunit that were recognized by intestinal secretory immunoglobulin A (IgA) antibodies from immune human subjects. These epitopes are contained in the region encompassing amino acids 758 to 1134 of the lectin heavy subunit, designated LC3. Baboons (Papio anubis) are natural hosts for Entamoeba histolytica; naturally infected baboons raised in captivity possess serum IgA antibodies to the same four LC3 epitopes as humans. Uninfected, seronegative baboons received four intranasal immunizations at 7-day intervals with the synthetic peptide vaccine (400, 800, or 1,600 μg per nostril) with cholera toxin (20 μg) as the adjuvant. As determined by an enzyme-linked immunosorbent assay (ELISA), each dose of the peptide vaccine elicited antipeptide serum IgA and IgG and intestinal IgA antibody responses in all six immunized baboons by day 28, 7 days after the last immunization (P, <0.01 for each dose compared to the cholera toxin control). The peptide vaccine elicited serum IgG and intestinal IgA antibodies that recognized purified recombinant LC3 protein (P, <0.008 and 0.02, respectively) and native lectin protein (P < 0.01). In addition, an indirect immunofluorescence assay with whole trophozoites (P < 0.01) and Western blot analysis confirmed that serum IgG antibodies from vaccinated baboons recognized native lectin protein on the surfaces of axenic E. histolytica trophozoites or from solubilized amebae. All four synthetic peptides were immunogenic; the vaccine elicited dose- and time-dependent responses, as determined by ELISA optical density readings indicating the production of serum and intestinal antibodies (P, <0.02 for antipeptide and antilectin antibodies). As a positive control, intranasal immunization with purified recombinant LC3 protein with cholera toxin as the adjuvant elicited a serum anti-LC3 IgA and IgG antibody response (P, 0.05 and <0.0001, respectively); however, no intestinal anti-LC3 IgA antibody response was observed (P = 0.4). Of interest, serum IgA and IgG antibodies elicited by the recombinant LC3 vaccine did not recognize any of the four putatively protective LC3 peptide epitopes. Both serum and fecal antibodies elicited by the peptide vaccine exhibited neutralizing activity, as determined by their dose-dependent inhibition of the galactose-specific adherence of E. histolytica trophozoites to Chinese hamster ovary cells in vitro (P, <0.001 for each group of antibodies compared to the control). In summary, a lectin-based intranasal polylysine-linked synthetic peptide vaccine was effective in eliciting an adherence-inhibitory, intestinal antilectin IgA antibody response in baboons. Future studies with the baboon model will determine vaccine efficacy against asymptomatic E. histolytica intestinal infection.

Invasive amebiasis, due to the enteric protozoan Entamoeba histolytica, manifests most commonly as amebic colitis or a liver abscess. E. histolytica infection is highly endemic in the most populated areas of the world, such as India, China, Africa, the Middle East, South and Central America, and southeast Asia (35). Recent epidemiological studies in Hue, Vietnam (8), South Africa (1, 36), and Bangladesh (20) revealed that E. histolytica infection and invasive disease are more prevalent than previously appreciated. Up to 70% of adults in an area where infection is highly endemic, such as Durban, South Africa, are challenged by the parasite each year (1); the prevalence of amebic liver abscesses (ALA) is as high as 21 per 100,000 people per year in Vietnam (8). It is estimated that E. histolytica is the third leading parasitic cause of death worldwide (43).

Presently, no vaccine to prevent asymptomatic E. histolytica infection or invasive disease is available. E. histolytica trophozoites attach to colonic mucins, host inflammatory cells, and epithelial cells by a galactose-inhibitable surface lectin (10, 32, 37). The lectin consists of three subunits (16); the heavy subunit contains the galactose-binding activity (33) and is highly antigenic (38). Purified native lectin protein (33) and a cysteine-rich recombinant protein encompassing amino acids 758 to 1134 of the lectin heavy subunit (designated LC3) (40) are effective as a vaccine (in combination with Freund’s adjuvant) in the gerbil model of ALA. Monoclonal antibodies to the lectin heavy subunit inhibit amebic binding to colonic mucins (10), suggesting that an intestinal antilectin immunoglobulin A (IgA) antibody response may be effective in preventing parasite attachment to and invasion of the gut. Studies of asymptomatic E. histolytica intestinal infection in children in Bangladesh (19–21) and in adults in Durban, South Africa (1, 36),

* Corresponding author. Mailing address: Department of Medicine, University of Minnesota, 14-110 Phillips Wangensteen Building, 516 Delaware St., S.E., Minneapolis, MN 55455. Phone: (612) 625-3654. Fax: (612) 626-3055. E-mail: Ravdi001@umn.edu.

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demonstrated that intestinal antilectin IgA antibodies mediate the rapid clearance of infection or immunity to new infections. Studies in Durban, South Africa, by our research group (1, 36) revealed that subjects cured of ALA develop high-titer peaks of intestinal antilectin IgA antibodies upon parasite challenge and that these antibody levels are sufficient to prevent or clear asymptomatic E. dispar and E. histolytica infections. In contrast, control subjects without a history of treatment for invasive amebiasis have lower-titer intestinal antilectin IgA antibody peaks that are delayed, less long-lasting, and insufficient to clear amebic infection (1).

Our strategy for vaccine design was to use synthetic peptides to elicit a mucosal immune response that mimics that of the South African ALA subjects. By using segments of the LC3 protein, expressed as overlapping recombinant proteins, we identified two LC3 fragments that are recognized by the antilectin IgA antibodies present in the sera and feces of immune human hosts (2). Fine mapping using overlapping synthetic peptides led to the discovery of four discrete LC3 peptide epitopes, referred to as peptides 2, 9, 11, and 12. Further studies have demonstrated that LC3 epitope recognition by human IgA antibodies is highly conserved and was exhibited by serum IgA antibodies from subjects cured of colitis in Cairo, Egypt (our unpublished data). Based on the epitope-mapping studies, we constructed a synthetic peptide vaccine containing the four putatively protective LC3 epitopes (peptides 2, 9, 11, and 12).

The systemic immunization of BALB/c mice with purified recombinant LC3 protein elicits murine IgA antibodies with LC3 epitope specificity different from that of human IgA antibodies, with little overlap (2). In addition, most mouse strains exhibit high levels of innate immunity to E. histolytica infection (22). Baboons were chosen for this vaccine study as they are naturally infected with E. histolytica in the wild (25, 42) and in captivity (45); in addition, baboons have an immune system that best approximates that of humans (39). Cholera toxin (CT) was selected as the adjuvant because of its known effectiveness in inducing a mucosal immune response (30) and because of our prior experience using recombinant LC3 protein to induce the secretion of intestinal adherence-inhibitory, antilectin IgA antibodies in BALB/c mice following oral immunization (7). Intranasal vaccination requires a smaller immunogen dose than oral vaccination (13) and obviates the need for sterile needles, an advantage considering the future application of a lectin-based subunit vaccine for humans.

Prior to being selected for the study, baboons were screened for E. histolytica and E. dispar infection by PCR analysis of fecal samples (2) and an enzyme-linked immunosorbent assay (ELISA) of serum samples for anti-LC3 IgA and IgG antibodies. Baboons were sedated with an intramuscular injection of ketamine hydrochloride (approximately 10 mg/kg of body weight) for the collection of blood using a 20-gauge needle and a 10-ml syringe. Fecal and blood samples were collected from baboons prior to each vaccination and 7 days after the last immunization. Blood was divided between a serum separator tube and a heparin tube and then placed on ice until centrifuged. After clotting for about 2 h, the samples were centrifuged for 15 min at high speed. The sera were stored at −80°C and shipped in dry ice to laboratories at the University of Minnesota.

**Detection of E. dispar or E. histolytica infection by fecal PCR assay.** Baboon stool samples were stored at −70°C, and extracted DNA was stored at −20°C in a fecal DNA bank. The QIAamp DNA stool mini kit was used to extract DNA from feces according to the protocol of the manufacturer (Qiagen, Halden, Germany). Four separate laboratory areas were used for PCR analysis to minimize the risk of contamination. DNA was extracted from stool in one area, and the PCR mix was prepared and samples were added in another area. The PCR was run in a third area, and the analysis of amplified PCR products and the storage of the PCR products and other materials (glassware and equipment) occurred in the remaining area.

PCR was performed using deoxynucleoside triphosphates (Amersham Pharmacia Biotech; catalog no. 27-2035-01) by mixing 100 μl of each nucleotide (G, C, T, and A) with 5 μl of PCR buffer and 4.6 ml of H2O. The mixture was divided into 1-ml aliquots and stored at −20°C. The Taq polymerase (Amersham Pharmacia Biotech; catalog no. 270799) was diluted at 1:20 immediately before use. The E. histolytica senser primer (5'-GTA AAT GGC CAA TCC ATC CAA CCA TGG TTT GTT-3') and the E. dispar sense primer (5'-GTA CAA AGT CCG CAT C-3') were prepared at concentrations of 10 Pmol/μl. A 500-μl volume of bovine serum albumin (BSA [Pierce; catalog no. 23210]) at 200 mg/ml was diluted with 500 μl of H2O and kept at 4°C. The DNA sample to be tested (5 μl) was added to 95 μl of the PCR mixture to yield a complete volume of 100 μl. Each DNA sample was tested twice, once using the E. histolytica sense primer and once using the E. dispers sense primer.

Conventional PCR machine thermocycling conditions were as described previously (2). The specific detection of amplified DNA was achieved by gel electrophoresis. Amplified DNA was separated on a 2% agarose gel containing ethidium bromide.

**ELISA for detection of baboon antilectin, anti-LC3, and antipeptide antibodies.** ELISAs were performed as described previously (36). Recombinant 52-kDa LC3 protein was purified as described previously (40), and native lectin protein was purified by immunoaffinity chromatography from detergent-solubilized trophozoites (33, 40). ELISA 96-well microtiter flat-bottomed polystyrene plates were coated with LC3 protein, purified native lectin, or synthetic peptides, and nonreactive sites were blocked with 1% BSA. Serum samples at a 1:100 dilution in phosphate-buffered saline (PBS)-TWEEN (1% BSA) were analyzed for IgG or IgA antibodies. Each sample was incubated for 2 h at room temperature or overnight at 4°C. Each sample was studied in triplicate and tested twice by ELISAs. Alkaline phosphatase-conjugated goat anti-human IgG (Sigma, St. Louis, MO) or IgA (ICN Biomedicals, Costa Mesa, CA) antibodies were diluted (1:5,000 for IgG and 1:2,500 for IgA) in PBS-TWEEN (1% BSA) to give a final volume of 100 μl/well, and the plates were incubated for 2 h at room temperature. Reading of the plates and correction for nonspecific background binding were performed as described previously (38).

ELISAs for the detection of fecal antilectin IgA antibodies were performed using purified native lectin protein as described previously (1, 3). We have previously reported that native lectin is more sensitive than recombinant LC3 protein for the detection of antilectin intestinal IgA antibodies in humans (3, 36). Flat-bottomed microtiter ELISA plates were coated with lectin protein (0.1 μg/well), and nonreactive sites were blocked with 1% BSA (3). Fecal samples were mixed with equal volumes of PBS containing 2.0 mM phenylmethylsulfonyl fluoride, and the mixtures were added at 100 μl/well and incubated for 2 h at room temperature or overnight at 4°C. Alkaline phosphatase-conjugated goat anti-human IgA antibodies (Sigma, St. Louis, MO) diluted (1:3,000) in PBS-TWEEN containing 1% BSA were added for incubation over 2 h at room temperature. Plate reading with correction for nonspecific background binding was performed as described previously (3).

**Preparation of the polylysine-linked synthetic peptides for use in the intranasal vaccine.** Based on previous findings (2), four peptides that included amino acids 891 to 903 (peptide 2), 918 to 936 (peptide 9), 1114 to 1138 (peptide 11), and 1128 to 1150 (peptide 12) of the LC3 protein were utilized. Peptides were synthesized using a PerkinElmer Pioneer peptide synthesizer by solid-phase materials and methods

Selection of experimental subjects (baboons). Female baboons, aged 1.5 and 3 years, were selected to participate in this study. Before being selected for the study, baboons (Papio anubis) of both sexes and various ages were housed in large breeding groups at the University of Oklahoma Health Sciences Center Comparative Medicine facility (which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International). Baboons were not utilized in other experiments before this study. During the immunization experiment, baboons were housed singly in aluminum cages. All experimental procedures were approved by the University of Oklahoma Health Sciences Center and the University of Minnesota Institutional Animal Care and Use Committees. Baboons were fed monkey chow twice daily and given fresh fruit, popcorn, peanuts, and other treats once a day. Study baboons did not show any physical or behavioral abnormalities before selection. Vaccinated and control animals lived under identical conditions and were managed by the same personnel throughout the study.

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results

We screened a total of 131 baboons from the colony and found that 16% were positive for serum antigen IgG antibodies, 6.5% were positive for serum antigen IgA antibodies, and 9.9% were positive for intestinal anti-lectin IgA antibodies. None of the baboons screened by fecal PCR were infected with E. histolytica; however, 22% were infected with E. dispar. We found that antilectin intestinal IgA antibodies from baboons naturally infected in captivity recognized all of the LC3 peptide epitopes included in the vaccine (optical density [OD] readings were 2.76, 2.08, 0.393, and 0.494 for LC3 peptides 2, 9, 11, and 12, respectively). Baboons selected for the study were seronegative and were not infected with E. histolytica or E. dispar.

Antibody responses to the synthetic peptide and recombinant LC3 vaccines were examined by three separate ELISAs using either all four synthetic peptides, purified recombinant LC3 protein, or immunoaffinity chromatography-purified native lectin (obtained from solubilized trophozoites) as the capture antigen. We found that baboons immunized intranasally with the synthetic peptide vaccine demonstrated brisk serum antipeptide IgA, serum antipeptide IgG, and intestinal anti-lectin IgA antibody responses in a dose-dependent fashion (P < 0.03 for each type of response compared to those in control baboons) (Fig. 1A, B, and C, respectively). Higher doses of the peptide vaccine (800 and 1,600 g) elicited a more rapid (P < 0.02) and incrementally greater antibody response than the 400-g dose (P < 0.003 for each antibody studied).
Of interest, by day 28, 100% of the six baboons studied for each peptide vaccine dose (a total of 18 baboons) developed antipeptide serum IgA and serum IgG antibody responses (*P* < 0.01 for each group compared to controls); at the 800- and 1,600-µg doses, 100% of the baboons had intestinal antipeptide IgA antibody responses by 14 and 21 days after immunization (*P* < 0.02 compared to the control), but not at the 400-µg dose (*P* = 0.26), induced an intestinal IgA antibody response to the peptide as measured over 4 weeks. However, at day 28, positive responses to all doses were observed (*P* < 0.02 compared to the control), and dose-dependent responses were observed over the 4 weeks (*P* = 0.004). The LC3 vaccine did not elicit any serum IgA (A) or IgG (B) antibodies to the peptide antigen (*P* = 0.80) compared to the control. The LC3 vaccine did not induce any intestinal antipeptide IgA antibody response (*P* = 0.46) (C).

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after the fourth immunization with the 400- and 1,600-μg peptide doses \((P < 0.02)\) (Fig. 2C). The recombinant LC3 vaccine induced high ODs indicating high levels of serum IgA and IgG antibodies to itself \((P, <0.001 \text{ compared to controls})\) (Fig. 2A and B). However, no measurable intestinal IgA antibody response was elicited by the LC3 vaccine, even at 28 days (Fig. 2C) \((P, <0.001 \text{ compared to the peptide vaccine and } >0.5 \text{ compared to saline controls})\).

Lastly, we studied antibody responses using immunoaffinity chromatography-purified native lectin as the capture antigen in the ELISA. Serum IgA and IgG antibodies elicited by the peptide vaccine reacted with immunoaffinity chromatography-purified lectin, but at low levels as indicated by ODs (Fig. 3). However, the synthetic peptide at two doses (400 and 1,600 μg) elicited intestinal IgA antibodies in a dose-dependent manner \((P = 0.03)\), and after four vaccinations, these antibodies demonstrated high levels of recognition of purified native lectin protein \((P, <0.01 \text{ compared to those from control baboons at 28 days})\) (Fig. 3C). Lastly, vaccination with the recombinant LC3 protein elicited serum IgA and IgG antibodies to immu-
no affinity chromatography-purified lectin protein at high levels as indicated by OD readings ($P < 0.001$ compared to the control) (Fig. 3A and B). However, no significant intestinal antilectin IgA antibody response was observed at 28 days after four intranasal vaccinations with LC3 protein (Fig. 3C) ($P = 0.80$).

To determine whether the low level of recognition in the ELISA of immunoaffinity chromatography-purified lectin protein by peptide vaccine-elicited serum IgA and IgG antibodies compared to recombinant LC3 protein-elicited antibodies may have been due to the alteration of the protein epitopes during the detergent solubilization and protein purification process, we performed IFAs using intact axenic *E. histolytica* trophozoites. Serum IgG antibodies from peptide (1,600-$\mu$g-dose)-immunized baboons were reactive to trophozoite surface antigen at serial serum dilutions from 1/8 to 1/128 as evaluated by an IFA ($P = 0.002$) for each dilution compared to sera from control baboons (Fig. 4). In addition, every baboon immunized with the peptide vaccine demonstrated a positive antilectin

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**FIG. 3.** Effects of intranasal immunization of baboons with the polylysine-linked synthetic peptide vaccine (400, 800, and 1,600 $\mu$g), the recombinant LC3 protein vaccine (200 $\mu$g), or CT adjuvant (20 $\mu$g) alone as assessed by an ELISA using immunoaffinity chromatography-purified galactose-inhibitable lectin as the capture antigen. The synthetic peptide vaccine did not elicit significant serum antilectin IgA antibodies (A) as determined by the ELISA ($P > 0.11$). There was a statistically significant but meager serum antilectin IgG antibody response (B) at the 1,000-$\mu$g dose ($P = 0.003$ over 4 weeks and 0.0004 at 4 weeks compared to the control). Of greatest interest, the synthetic peptide vaccine at the 400- and 1,600-$\mu$g doses elicited an intestinal antilectin IgA response (C) compared to the control after 4 weeks ($P = 0.004$). There was a positive dose-dependent response ($P = 0.0002$) and an increase in the ELISA OD reading over time ($P = 0.002$). The LC3 vaccine elicited substantial serum antilectin IgA ($P = 0.02$) (A) and IgG ($P < 0.0002$) (B) antibody responses over time and at 4 weeks. In contrast, the LC3 vaccine did not induce any intestinal antilectin IgA response (C) by 28 days ($P = 0.80$).
antibody response as determined by the IFA (\( P < 0.001 \) for the 1/8 dilution compared to the controls). To correlate with the ELISA and IFA results, we found by Western blot analysis that serum IgG antibodies from peptide-immunized baboons recognized the lectin 170-kDa heavy subunit to be present in whole axenic trophozoites better than in immunoaffinity chromatography-purified native lectin (Fig. 5C and D). As a positive control, immune human sera from ALA subjects demonstrated recognition of the 170-kDa lectin subunit in whole trophozoites and in the immunoaffinity chromatography-purified lectin protein (Fig. 5C and D). Control human and baboon sera were nonreactive (Fig. 5A and B).

To determine which of the peptides utilized in the vaccine were immunogenic, we preformed ELISA peptide dose analysis for intestinal IgA and serum IgA and IgG antibodies to each of the four peptides by using pooled sera and feces from baboons immunized at the 1,600-µg peptide dose (Table 1). We found that LC3 peptides 2, 9, and 12 elicited intestinal and serum IgA antibody responses; LC3 peptides 9, 11, and 12 elicited serum IgG antibody responses (Table 1).

To determine whether serum and intestinal antilectin antibodies elicited by the peptide vaccine exhibited neutralizing activity against viable \( E. \) histolytica trophozoites, we studied galactose-specific adherence in vitro using the well-established ameba-CHO cell rosetting model (38). Trophozoites were incubated at 4°C with various dilutions of sera or fecal supernates, washed, and then pelleted with CHO cells to study adherence. A positive control with galactose and a negative control with test medium combined with sera or fecal supernates from control baboons (receiving adjuvant only) were studied. As expected, galactose exhibited a dose-dependent (0.05 to 0.4%) inhibition of trophozoite adherence to CHO.
TABLE 1. Recognition of individual peptides by serum and intestinal antibodies from vaccinated baboons

<table>
<thead>
<tr>
<th>Antibody group</th>
<th>OD reading for indicated LC3 peptide</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>2 (aa 891–903)</td>
</tr>
<tr>
<td>Fecal antipeptide IgA</td>
<td>1.829</td>
</tr>
<tr>
<td>Serum antipeptide IgA</td>
<td>0.904</td>
</tr>
<tr>
<td>Serum antipeptide IgG</td>
<td>0.518</td>
</tr>
<tr>
<td>Fecal antipeptide IgA</td>
<td>1.829</td>
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<tr>
<td>Serum antipeptide IgA</td>
<td>0.904</td>
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<tr>
<td>Serum antipeptide IgG</td>
<td>0.518</td>
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<sup>a</sup> Serum and fecal samples from the six baboons immunized with the 1,600-μg dose of polylysine-linked synthetic peptides were pooled and studied in duplicate.

<sup>b</sup> A positive result was defined as a reading ≥4-fold higher than the OD reading for control baboon sera. aa, amino acids.

In summary, we demonstrated that an intranasal synthetic peptide amebiasis subunit vaccine, which includes the four LC3 epitopes recognized by IgA antibodies from immune human subjects, with CT as the adjuvant, was highly immunogenic in baboons as determined by ELISAs for peptide-specific serum IgA, serum IgG, and intestinal IgA antibodies. The peptide vaccine elicited intestinal IgA antibodies that reacted strongly with immunofluorescence chromatography-purified native lectin protein as demonstrated by ELISA; peptide vaccine-elicited serum IgA or IgG antibodies exhibited low levels of reactivity to LC3 or immunofluorescence chromatography-purified lectin. To further address the recognition of lectin molecules by peptide vaccine-elicited antibodies, we performed IFAs and Western blot analysis and studied in vitro amebic adherence to CHO cells. IFAs demonstrated serum IgG antibody recognition of lectin molecules on the surfaces of axenic trophozoites, and immunoblotting demonstrated the recognition of the lectin heavy-subunit protein present in axenic lysates. Therefore, the lower level of reactivity of peptide vaccine-elicited serum antibodies than of recombinant LC3 protein-elicited antibodies to purified lectin protein as evaluated by ELISAs may have been due to the alteration of the peptide epitopes on the native lectin molecule by the detergent solubilization and immunopurification process. The recombinant LC3 protein, as a positive control, was effective in eliciting serum IgG and IgA antibodies to itself and to purified native lectin protein. However, the recombinant LC3 vaccine did not elicit a mucosal immune response. In addition, the serum anti-LC3 IgG and IgA antibodies elicited by the LC3 vaccine did not recognize the four peptide epitopes recognized by serum and intestinal IgA antibodies from immune human subjects (2). Therefore, recombinant LC3 protein would not be suitable for use as an intranasal subunit vaccine to elicit a protective mucosal immune response. Importantly, the synthetic peptide vaccine elicited neutralizing serum and fecal antilectin antibodies as evidenced by a dose-dependent inhibitory effect on the galactose-specific in vitro adherence of viable E. histolytica trophozoites to CHO cells.

To our knowledge, this is the first experimental study of an intranasal synthetic peptide amebiasis subunit vaccine. Investigators from our laboratory were also the first to report that a purified E. histolytica protein, the galactose-inhibitable lectin, is highly efficacious in a gerbil model of ALA (33). Gerbils immunized with native lectin protein exhibited a protective efficacy of 70% against intrahepatic challenge with axenic trophozoites; however, a subset of vaccinated animals developed larger abscesses than controls (33). Concern that vaccination with purified lectin may induce adherence-enhancing antibodies (34) or be immunosuppressive were raised. Therefore, the need for a more defined amebiasis subunit vaccine became evident. We subsequently reported that purified recombinant LC3 protein under the same experimental conditions was equally effective in the gerbil ALA model (40) without any paradoxical worsening of disease in those immunized (40). The gerbil ALA model has been utilized for the study of numerous amebiasis subunit vaccines, including native and recombinant forms of the galactose-inhibitable lectin (34), the EhADHI112 protein (29), and recombinant Yersinia enterocolitica expressing hybrid type III proteins containing the cysteine-rich section of the lectin heavy subunit (26). A DNA vaccine expressing the serine-rich protein SREHP (45) and a 24-mer derived from the lectin heavy subunit chemically coupled to keyhole limpet hemocyanin (27) are also protective in experimental gerbil or hamster liver abscess models. However, immunity to experimental ALA is dependent on cellular rather than mucosal immune mechanisms (24), bypasses the natural gastrointestinal route of infection, and thus is not analogous to the study of asymptomatic intestinal infection in humans.

In a susceptible-mouse (C3H) model, the galactose-inhibitable lectin and, to a lesser extent, a 64-kDa fragment of the lectin heavy subunit with Freund’s adjuvant provide protection against experimental cecal inoculation (22). Studies with resistant mouse strains indicated that neutrophil and innate immune mechanisms are protective (5). Passive transfer with antibodies to the amebic proteophosphoglycan, an abundant surface molecule, prevented colitis in human intestinal xenografts placed in SCID mice (46). As mice are not natural hosts of E. histolytica and have vaccine immunity mediated by...
secretory IgA-independent mechanisms and epitope recognition differing from that observed following human infection (2, 40), we chose to use a primate model.

Baboons get infected with *E. histolytica* and *E. dispar* in the wild and in captivity (23, 25, 42) and are susceptible to amebic colitis (25, 42). They have the same immunoglobulin subclasses as humans (39). Upon the screening of a large, asymptomatic baboon colony, we found that antilectin IgA antibodies from

FIG. 6. Inhibition of amebic galactose-specific adherence to CHO cells by sera (A) or fecal supernates (B) from peptide (1,600-μg-dose)-immunized or control baboons. Axenic *E. histolytica* trophozoites were incubated at 4°C with or without increasing concentrations of galactose, sera, or fecal supernates. As expected, increasing concentrations of galactose demonstrated a dose-dependent inhibition of ameba-CHO cell rosette formation (*P* <0.01 for each concentration compared to control media). Sera from peptide-immunized baboons (*P* <0.01 at each dilution) and sera from baboons receiving adjuvant only (*P* <0.001 at each comparable dilution) demonstrated significant inhibition of amebic galactose-specific adherence compared to control media. The adherence-inhibitory effects of immune baboon sera were no different from those of galactose. Fecal supernates from peptide-immunized baboons inhibited amebic adherence to CHO cells compared to fecal supernates from baboons receiving CT adjuvant only (*P* <0.01 at each dilution). The adherence-inhibitory effects of fecal supernates from immunized baboons were also concentration dependent (*P* <0.01 for each dilution compared to the proceeding one) and comparable to the adherence-inhibitory effects of galactose.
naturally infected baboons recognized the same four LC3 peptide epitopes as antibodies from seropositive humans, further confirming the suitability of baboons as an experimental vaccine host. The nasal route of vaccination offers many advantages, including easy access, lack of the need for sterile syringes, and the successful induction of mucosal and systemic immune responses, and requires lower doses of antigen than the oral route (13, 31). Antigen-specific B and T lymphocytes migrate from nose-associated lymphoid tissues to regional lymph nodes and distant effector sites (31, 45). Intranasal vaccination is more effective than the intragastric route in generating a rapid, retained, and strong mucosal immune response (44); mucosal immune responses in the gastrointestinal tract do occur after intranasal vaccination (11).


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