

AMEBIASIS SUBUNIT VACCINE**Claim of Priority**

This patent application claims benefit to U.S. Application Serial No. 60/581,248 filed on June 18, 2004, and U.S. Application Serial No. 60/581,577 filed on June 21, 2004. These applications are hereby incorporated by reference herein.

Statement Regarding Federally Sponsored Research Or Development

This work was supported by NIH grants PO1-AI36359-01 and UO1-AI35840 from NIAID. The government may have certain rights in the invention.

BACKGROUND

Amebiasis is a disease or infection caused by an enteric protozoan *Entamoeba histolytica*. *E. histolytica* are members of a large genus of naked rhizopod protozoans. *E. histolytica* is a parasitic amoeba of humans that can cause breakdown of body tissues during infection. *E. histolytica* is extremely common. Worldwide, in endemic areas such as India, Africa, Asia, Mexico, and South America, up to 20% of the population is infected each year. Of those with infections, only about 10% develop symptoms such as colitis or liver abscess. The low incidence of symptoms is thought to be due to a balance between host immune surveillance, parasite strain variation, and local factors such as diet or bacterial flora. The "pathogenic form" of amebic intestinal infection refers to *E. histolytica* which may be invasive and result in symptoms in infected subjects and elicits a systemic immune regime. "Nonpathogenic form" of amebiasis refers to *Entamoeba dispar*. *E. dispar* is morphologically identical to *E. histolytica* but is genetically a distinct species, which is harbored asymptotically by carriers of the infection. Subjects who eventually exhibit symptoms harbor *E. histolytica*, which was first established on the basis of their distinctive hexokinase and phosphoglucumutase isoenzymes, and later on the basis of antigen specificity in ELISA or by genetic differences by PCR. It is known that *E. histolytica* infection is mediated at least in part by the "Gal/GalNAc" adherence lectin. The purified lectin was shown to have a non-reduced molecular weight of 260 kDa on SDS-PAGE; reduction with β -

mercaptoethanol yielded two subunits having molecular masses of 170 kDa (heavy subunit) and 35 kDa (light subunit). Antibodies directed to the 170 kDa subunit were capable of blocking surface adhesion to test cells (Petri, 1989). Therefore, the 170 kDa subunit is believed to be of primary importance in mediating adhesion, and is at times referred to as the 170 kDa adhesin. DNA molecules encoding both the heavy and light subunits have been cloned. The heavy and light subunits are encoded by distinct mRNAs (Mann, 1991), and these subunits have different amino acid compositions and N-terminal sequences.

SUMMARY OF THE INVENTION

The present invention provides an *Entamoeba histolytica* lectin-based, LC3 subunit peptide vaccine, wherein the LC3 subunit peptide is present in an effective amount to induce mucosal anti-lectin IgA antibody production in a primate (*e.g.*, a human) against *Entamoeba histolytica* or *E. dispar*. In certain embodiments, the LC3-based subunit peptides that are used in the vaccine include portions of epitope 3 or epitope 7 of *E. histolytica* LC3 recombinant protein, which is a cysteine-rich protein of the lectin heavy subunit (amino acids 758-1134, numbering scheme consistent with Tannich, 1991). For example, the *E. histolytica* LC3 subunit peptide vaccine is encoded by LC3 amino acids numbered 891-903 or 918-936, which are subunits of epitope 3 and by amino acids numbered 1114-1128 or 1128-1150, which are subunits of epitope 7. The vaccine may be in combination with a physiologically-acceptable, non-toxic vehicle and adjuvant.

In certain embodiments, the LC3 subunit peptide is expressed from an isolated DNA sequence encoding the LC3 subunit peptide. In certain embodiments, the DNA encodes an LC3 subunit peptide containing contiguous amino acid residues from about residue 891 to about residue 903, from about residue 918 to about residue 936, from about residue 1114 to about residue 1128, or from about residue 1128 to about residue 1150. In certain embodiments, the LC3 subunit peptide is a variant of wild-type LC3 subunit peptide in that the variant LC3 subunit peptide has a modification at one or more amino acid residues. The modification may, for example, be a substitution at one or more amino acid residues (*e.g.*, a conserved substitution). The vaccine may further contain an effective amount of an immunological adjuvant or other immune

stimulating agent. In certain embodiments, at least one subunit peptide is conjugated or linked to a carrier molecule. For example, one, two, three, four, five, or six peptides can be linked to a carrier molecule. These peptides can all be the same type of peptide, or can be different peptides. For instance, three
5 subunit peptides containing amino acid residues 918-936 and three subunit peptides containing amino acid residues 1128-1150 may all be linked to a polylysine backbone. In another example, at least one of all four possible peptides is linked to the carrier molecule. The carrier molecule may, for example, be a polypeptide or a polysaccharide.

10 The present invention also provides a method of protecting a susceptible primate (*e.g.*, a human) against *E. histolytica* or *E. dispar* colonization or infection by administering to the primate an effective amount of a vaccine containing an *Entamoeba histolytica* LC3 subunit peptide, wherein the LC3 subunit peptide is present in an effective amount to induce mucosal anti-lectin
15 IgA antibody production in the primate, and wherein the LC3 subunit peptide is in combination with a physiologically-acceptable, non-toxic vehicle. In certain embodiments, the vaccine is administered by subcutaneous or intramuscular injection. In other embodiments, the vaccine is administered by oral ingestion or intranasally.

20 The present invention further provides an isolated and purified *Entamoeba histolytica* LC3 subunit peptide, wherein the LC3 subunit peptide induces mucosal anti-lectin IgA antibody production in a primate against *Entamoeba histolytica* or *E. dispar*. In certain embodiments, the subunit peptide is *Entamoeba histolytica* epitope 3 (amino acids 868-944) or epitope 7 (amino
25 acids 1114 to 1134). For example, the subunit peptide is encoded by LC3 amino acids numbered 891-903, 918-936, 1114-1128, or 1128-1150.

The present invention also provides an isolated and purified polynucleotide containing a nucleotide sequence encoding an *E. histolytica* LC3 subunit peptide, wherein the LC3 subunit peptide induces mucosal anti-lectin
30 IgA antibody production in a primate against *E. histolytica* or *E. dispar*. The polynucleotide may be either DNA or RNA.

The present invention also provides a purified antibody that specifically recognizes an LC3 subunit peptide that comprises contiguous amino acid residues from about residue 891 to about residue 903, from about residue 918 to
35 about residue 936, from about residue 1114 to about residue 1128, or from about

residue 1128 to about residue 1150. The antibody may be monoclonal, polyclonal and/or humanized. In further aspects of the present invention, the invention is directed to both nucleic acid and immunological reagents that can be produced in view of the discovery of these peptides. These reagents are specific
5 for each of the nucleic acids that encode the peptides, as well as their RNA or protein products. For example, oligonucleotide probes specific for any one of these four peptides may be identified by one of ordinary skill in the art, using conventional nucleic acid probe design principles, by comparisons of the DNA sequences for these peptides.

10 Also provided is a method for immunizing a subject against *Entamoeba histolytica* or *E. dispar* infection by administering to the subject an effective amount of a vaccine as described above. Vaccination with the vaccine will result in an immune response that blocks the carbohydrate-binding activity of the amoeba, which is needed for both colonization and host cell killing.

15 The present invention also provides methods of identifying further subunit peptides, using the techniques described in the Examples below.

DESCRIPTION OF DRAWINGS

Figure 1. Recombinant protein fragments produced from restriction enzyme digestion of LC3 DNA. Restriction enzymes sites are indicated,
20 fragments are represented by their amino acid sequence numbers. Seven recombinant LC3 protein fragments (A through G) were produced.

Figure 2. ELISA OD results for serum anti-LC3 IgA (Figure 2A) and IgG antibodies (Figure 2B) using purified LC3 antigen (means indicated by horizontal bar). Study groups include seronegative uninfected controls,
25 asymptomatic, seropositive subjects with current *E. histolytica* infection as determined by fecal PCR, subjects recently cured of ALA with and without infection (by PCR), and ALA subjects free of infection by culture criteria one year after cure. There were substantially higher IgA and IgG antibody levels in recently cured ALA subjects with or without infection and seropositive subjects
30 with asymptomatic intestinal infection, compared to controls and ALA subjects cured one year ago ($p < 0.05$ for each).

Figure 3. Recognition of LC3 epitopes by human anti-LC3 IgA and IgG antibodies and by anti-LC3 murine IgA monoclonal antibodies. By analysis of serum IgA and IgG antibody recognition of LC3 protein fragments, we found

that IgA antibodies from ALA subjects (infected or uninfected) and from seropositive subjects with asymptomatic *E. histolytica* infection all recognized epitopes 3 and 7 (shaded oval). Only anti-LC3 IgG antibodies recognized epitope six (open oval) which was lost only one year after cure of ALA. Anti-LC3 murine IgA monoclonal antibodies recognized epitopes 1, 2, 4, 5, and 6 (*) and not epitopes 2 or 7.

Figure 4. Recognition by pooled human intestinal and serum anti-LC3 IgA antibodies of synthetic peptides based on the amino acid sequences of LC3 epitope 3 and 7. Ten overlapping peptides were synthesized based on the sequence of epitope 3 and two peptides based on epitope 7. Intestinal (triangles) and serum (circles) IgA antibodies were obtained from subjects cured of ALA with current infection (black); seropositive, asymptomatic PCR (+) subjects (gray) and seronegative PCR (-) control subjects (open symbols). Peptides 2, 9, 11, and 12 were recognized by intestinal and serum IgA antibodies from all subjects, compared to the cutoff point established by seronegative controls. Peptides 2, 9, and 11 were recognized by serum IgA antibodies from ALA subjects and fecal and serum IgA antibodies from asymptomatic infected seropositive subjects.

Figure 5. A graph demonstrating serum anti-LC3 IgG antibody response in baboons on days 7, 14, 21, and 28 following four intranasal vaccinations on days 1, 7, 14, and 21 with cholera toxin (20 µg), adjuvant alone (black bar), LC3-based peptide vaccine (amino acids 891-903, 918-936, 1114-1138, 1128-1150 linked randomly, six molecules to a polylysine backbone; 200µg) (light bar in middle), or purified 52 kdalton LC3 recombinant protein (200 µg) plus cholera toxin (20 µg) (shaded bar). Both the intranasal recombinant LC3 vaccine and the synthetic peptide vaccine induced serum IgG antibodies to the recombinant LC3 protein as determined by ELISA.

Figure 6. A graph showing serum anti-lectin IgG antibody responses in baboons on days 7, 14, 21, and 28 following four intranasal vaccinations on days 1, 7, 14, and 21 with cholera toxin (20 µg), adjuvant alone (black bar), LC3-based peptide vaccine (amino acids 891-903, 918-936, 1114-1138, 1128-1150 linked randomly, six molecules to a polylysine backbone; 200µg) (light bar in middle), or purified 52 kDa LC3 recombinant protein (200 µg) plus cholera toxin (20 µg) (shaded bar). Importantly, both the recombinant LC3 protein and the synthetic peptide vaccine induced serum IgG antibodies that recognized

native Gal/GalNAC lectin from *E. histolytica* trophozoites, which is present on the surface of the parasite.

Figure 7. A graph showing serum anti-peptide IgG antibody responses to in baboons on days 7, 14, 21, and 28 following four intranasal vaccinations on days 1, 7, 14, and 21 with cholera toxin (20 µg), adjuvant alone (black bar), LC3-based peptide vaccine (amino acids 891-903, 918-936, 1114-1138, 1128-1150 linked randomly, six molecules to a polylysine backbone; 200µg) (light bar in middle), or purified 52 kDa LC3 recombinant protein (200 µg) plus cholera toxin (20 µg) (shaded bar). Only the synthetic peptides vaccine induced serum IgG antibodies to the four putatively protective LC3 epitopes, as determined by ELISA with purified peptides (amino acids 891-903, 918-939, 1144-1138, and 1128-1150). The recombinant LC3 vaccine induces IgG antibodies to itself (Figure 5) and the native lectin (Figure 6) but not to the four putatively protective LC3 epitopes defined by mapping with human IgA antibodies.

Figure 8. A graph showing the serum anti-LC3 IgA antibody response in baboons on days 7, 14, 21, and 28 following four intranasal vaccinations on days 1, 7, 14, and 21 with cholera toxin (20 µg), adjuvant alone (black bar), LC3-based peptide vaccine (amino acids 891-903, 918-936, 1114-1138, 1128-1150 linked randomly, six molecules to a polylysine backbone; 200µg) (light bar in middle), or purified 52 kDa LC3 recombinant protein (200 µg) plus cholera toxin (20 µg) (shaded bar). Only the recombinant LC3 protein vaccine induced a serum anti-LC3 IgA antibody response, none was observed with the synthetic peptide vaccine.

Figure 9. A graph showing serum anti-lectin IgA antibody response in baboons on days 7, 14, 21, and 28 following four intranasal vaccinations on days 1, 7, 14, and 21 with cholera toxin (20 µg), adjuvant alone (black bar), LC3-based peptide vaccine (amino acids 891-903, 918-936, 1114-1138, 1128-1150 linked randomly, six molecules to a polylysine backbone; 200µg) (light bar in middle), or purified 52 kDa LC3 recombinant protein (200 µg) plus cholera toxin (20 µg) (shaded bar). As in Figure 8, the recombinant LC3 vaccine, but not the synthetic peptide vaccine, induced serum anti-lectin IgA antibody response.

Figure 10. A graph showing serum anti-peptide IgA antibody response in baboons on days 7, 14, 21, and 28 following four intranasal vaccinations on days 1, 7, 14, and 21 with cholera toxin (20 µg), adjuvant alone (black bar),

LC3-based peptide vaccine (amino acids 891-903, 918-936, 1114-1138, 1128-1150 linked randomly, six molecules to a polylysine backbone; 200 μ g) (light bar in middle), or purified 52 kDa LC3 recombinant protein (200 μ g) plus cholera toxin (20 μ g) (shaded bar). Consistent with the results of LC3 recombinant vaccine induced serum IgG antibodies (Figure 7), recombinant LC3 protein vaccine (Figures 8 and 9) did not elicit serum IgA antibodies that recognize the four peptide epitopes; the synthetic peptide vaccine elicited a weak serum anti-peptide IgA antibody response.

Figure 11. A graph of fecal anti-LC3 IgA antibody response in baboons on days 7, 14, 21, and 28 following four intranasal vaccinations on days 1, 7, 14, and 21 with cholera toxin (20 μ g), adjuvant alone (black bar), LC3-based peptide vaccine (amino acids 891-903, 918-936, 1114-1138, 1128-1150 linked randomly, six molecules to a polylysine backbone; 200 μ g) (light bar in middle), or purified 52 kDa LC3 recombinant protein (200 μ g) plus cholera toxin (20 μ g) (shaded bar). As is evident, only the synthetic peptide vaccine induced a fecal (intestinal) anti-LC3 IgA antibody response; the recombinant LC3 protein vaccine induced serum anti-LC3 IgA antibodies (Figure 8) but not a fecal or mucosal anti-LC3 IgA antibody response.

Figure 12. A graph showing fecal anti-lectin IgA antibody response in baboons on days 7, 14, 21, and 28 following four intranasal vaccinations on days 1, 7, 14, and 21 with cholera toxin (20 μ g), adjuvant alone (black bar), LC3-based peptide vaccine (amino acids 891-903, 918-936, 1114-1138, 1128-1150 linked randomly, six molecules to a polylysine backbone; 200 μ g) (light bar in middle), or purified 52 kDa LC3 recombinant protein (200 μ g) plus cholera toxin (20 μ g) (shaded bar). Importantly, the synthetic peptide vaccine induced intestinal IgA antibodies that recognize the amebic native Gal/Gal/NAC lectin molecule present on the surface of the parasite; again, no mucosal IgA antibody response was observed with the LC3 recombinant protein vaccine.

Figure 13. A graph showing fecal anti-peptide IgA antibody response in baboons on days 7, 14, 21, and 28 following four intranasal vaccinations on days 1, 7, 14, and 21 with cholera toxin (20 μ g), adjuvant alone (black bar), LC3-based peptide vaccine (amino acids 891-903, 918-936, 1114-1138, 1128-1150 linked randomly, six molecules to a polylysine backbone; 200 μ g) (light bar in middle), or purified 52 kDa LC3 recombinant protein (200 μ g) plus cholera toxin (20 μ g) (shaded bar). As expected, only the synthetic peptide vaccine

induced mucosal IgA antibodies to the four putatively protective LC3 peptide epitopes; no response was observed with the LC3 protein vaccine.

DETAILED DESCRIPTION

Immunity to *Entamoeba* species intestinal infection is associated with the presence of intestinal IgA antibodies to the parasite's galactose-inhibitable adherence lectin. The inventors determined the epitope specificity of serum and intestinal (*i.e.*, mucosal) anti-lectin IgA antibodies by ELISA using overlapping fragments of a recombinant portion of the lectin heavy subunit, designated LC3. These findings were correlated with the effects of epitope-specific murine anti-lectin IgA monoclonal antibodies (MoAbs) on amebic *in vitro* galactose-specific adherence. LC3 is a highly antigenic and immunogenic cysteine-rich protein (AA 758-1134) that includes the lectin's carbohydrate binding domain. The study subjects, from Durban, South Africa, were recently cured of amebic liver abscess (ALA) with or without current *E. histolytica* intestinal infection or were infection-free one year after cure. The inventors also studied seropositive subjects that were currently infected with *E. histolytica*, disease-free, and asymptomatic. Serum anti-LC3 IgA antibodies from all study groups exclusively recognized the third (AA 868-944) and the seventh (AA 1114-1134) LC3 epitopes regardless of clinical status. Epitope six (AA 1070-1114) was also recognized by serum anti-LC3 IgG antibodies. However, IgG antibody recognition of epitope six, but not three or seven, was lost one year following cure of ALA. The inventors produced fourteen murine anti-LC3 IgA MoAbs that collectively recognize five of the seven LC3 epitopes. The majority of the murine MoAbs recognized the first epitope (AA 758-826), which was not recognized by human IgA antibodies. Interestingly, adherence of *E. histolytica* trophozoites to CHO cells was inhibited by MoAbs to epitopes one, three, four (AA 944-987), and six ($p < 0.01$). The LC3 epitopes recognized by human IgA antibodies (three and seven) were further characterized by use of overlapping synthetic peptides. The inventors identified four peptides (AA 891-903, 918-936, 1114-1138, and 1128-1150) that in linear or cyclized form were recognized by pooled intestinal IgA antibodies and serum IgG antibodies from ALA and asymptomatic, seropositive infected subjects. These lectin epitopes can be used in an amebiasis subunit vaccine designed to elicit mucosal immunity mimicking that of humans cured of ALA who are highly immune to new amebic infection

(Ravdin et al., 2003).

Vaccine Compositions

One or more subunits of the 170 kDa peptide are used as the active
5 ingredient of a vaccine composition. The present invention provides peptides
that are variants of LC3. A "variant" of LC3 is a polypeptide or oligopeptide
LC3 that is not completely identical to native LC3. Variant LC3 molecules
include peptides that are not full-length LC2 peptides, *i.e.*, are subunits of LC3.
Variant LC3 peptides can also be obtained by altering the amino acid sequence
10 by insertion, deletion or substitution of one or more amino acid. The amino acid
sequence of the protein is modified, for example by substitution, to create a
polypeptide having substantially the same or improved qualities as compared to
the native polypeptide. The substitution may be a conserved substitution. A
"conserved substitution" is a substitution of an amino acid with another amino
15 acid having a similar side chain. A conserved substitution would be a
substitution with an amino acid that makes the smallest change possible in the
charge of the amino acid or size of the side chain of the amino acid
(alternatively, in the size, charge or kind of chemical group within the side chain)
such that the overall peptide retains its spatial conformation but has altered
20 biological activity. For example, common conserved changes might be Asp to
Glu, Asn or Gln; His to Lys, Arg or Phe; Asn to Gin, Asp or Gli and Ser to Cys,
Thr or Gly. Alanine is commonly used to substitute for other amino acids. The
20 essential amino acids can be grouped as follows: alanine, valine, leucine,
isoleucine, proline, phenylalanine, tryptophan and methionine having nonpolar
25 side chains; glycine, serine, threonine, cystine, tyrosine, asparagine and
glutamine having uncharged polar side chains; aspartate and glutamate having
acidic side chains; and lysine, arginine, and histidine having basic side chains.

The amino acid changes are achieved by changing the codons of the
corresponding nucleic acid sequence. It is known that such polypeptides can be
30 obtained based on substituting certain amino acids for other amino acids in the
polypeptide structure in order to modify or improve antigenic or immunogenic
activity. For example, through substitution of alternative amino acids, small
conformational changes may be conferred upon a polypeptide that results in
increased activity or enhanced immune response. Alternatively, amino acid
35 substitutions in certain polypeptides may be used to provide residues that may

then be linked to other molecules to provide peptide-molecule conjugates which retain sufficient antigenic properties of the starting polypeptide to be useful for other purposes. In one embodiment a polylysine backbone may be used as a carrier for one or more of the same or different peptides of the present invention.

5 For example, six peptides are linked to a single polylysine backbone.

The LC3 peptide can be conjugated or linked to another peptide or to a polysaccharide. For example, immunogenic proteins well known in the art, also known as "carriers," may be employed. Useful immunogenic proteins include keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin, 10 human serum albumin, human gamma globulin, chicken immunoglobulin G and bovine gamma globulin. Alternatively, polysaccharides or proteins of other pathogens that are used as vaccines can be conjugated to, linked to, or mixed with the LC3 peptide such as tetanus toxoid.

One can use the hydropathic index of amino acids in conferring 15 interactive biological function on a polypeptide, wherein it is found that certain amino acids may be substituted for other amino acids having similar hydropathic indices and still retain a similar biological activity. Alternatively, substitution of like amino acids may be made on the basis of hydrophilicity, particularly where the biological function desired in the polypeptide to be generated is intended for use in immunological embodiments. The greatest local average hydrophilicity of 20 a "protein," as governed by the hydrophilicity of its adjacent amino acids correlates with its immunogenicity. Accordingly, it is noted that substitutions can be made based on the hydrophilicity assigned to each amino acid. In using either the hydrophilicity index or hydropathic index, which assigns values to each amino acid, the substitutions of amino acids are at values of ± 2 , such as at 25 ± 1 , or at ± 0.5 .

The variant LC3 peptide has at least 50%, at least about 80%, and at least about 90% or even 100% contiguous amino acid sequence homology or identity to the amino acid sequence of a corresponding native LC3.

30 The amino acid sequences of the LC3 peptides correspond essentially to portions of the native LC3 amino acid sequence. As used herein "correspond essentially to" refers to a polypeptide sequence that will elicit a protective immunological response. An immunological response to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated 35 immune response to the polypeptide or vaccine of interest. Usually, such a

response consists of the subject producing antibodies, B cell, helper T cells, suppressor T cells, and/or cytotoxic T cells directed specifically to an antigen or antigens included in the composition or vaccine of interest.

5 A variant of the invention may include amino acid residues not present in the corresponding native LC3 or deletions relative to the corresponding native LC3.

The LC3 peptides of the vaccine may be expressed from isolated DNA sequences encoding the LC3 peptides. "Recombinant" is defined as a peptide or nucleic acid produced by the processes of genetic engineering. The terms
10 "protein," "peptide" and "polypeptide" are used interchangeably herein.

Nucleic acid molecules encoding amino acid sequences encoding an LC3 peptide are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by
15 oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the LC3 peptide. Variant peptides may be conveniently prepared by direct chemical synthesis on solid phase supports and their subsequent separation from the support. Such methods are well known in the art.

20 A "chemical derivative" of the 170-kDa subunit contains additional chemical moieties not normally a part of the peptide. Covalent modifications of the peptide are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with
25 selected side chains or terminal residues.

Methods of Protecting a Subject from Infection

The present vaccine compositions are used for prevention of *E. histolytica* infection in subjects at risk for such an infection. Subunits of the
30 170-kDa protein can be used as immunogens. Since the epitope-bearing fragments are relatively short, for example containing 20 amino acids or less, it is advantageous to couple the peptide to an immunogenic carrier to enhance its immunogenicity. Such coupling techniques are well known in the art, and include standard chemical coupling techniques using linker moieties such as
35 those available from Pierce Chemical Company, Rockford, Ill. Suitable carriers

are proteins such as keyhole limpet hemocyanin (KLH), *E. coli* pilin protein k99, BSA, or rotavirus VP6 protein.

The LC3 peptide vaccine can be administered by subcutaneous or intramuscular injection. Alternatively, the vaccine can be administered by oral
5 ingestion or intranasal inoculation. To immunize a subject, the LC3 peptide, may be administered parenterally, usually by intramuscular or subcutaneous injection in an appropriate vehicle. Other modes of administration, however, such as oral delivery or intranasal delivery, are also acceptable. Vaccine formulations will contain an effective amount of the active ingredient in a
10 vehicle. The effective amount is sufficient to prevent, ameliorate, or reduce the incidence of *E. histolytica* colonization in the target mammal. An effective amount to be administered in a vaccine dose is readily determined by one skilled in the art. The active ingredient may typically range from about 1% to about 95% (w/w) of the composition, or even higher or lower if appropriate. The
15 quantity to be administered depends upon factors such as the age, weight, and physical condition of the animal or the human subject considered for vaccination. The quantity also depends upon the capacity of the animal's immune system to synthesize antibodies, and the degree of protection desired. Effective dosages can be readily established by one of ordinary skill in the art
20 through routine trials establishing dose response curves. The subject is immunized by administration of the LC3 peptide in one or more doses. Multiple doses may be administered as is required to maintain a state of immunity to *E. histolytica*.

Intranasal formulations may include vehicles that neither cause irritation
25 to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal
30 mucosa.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspension, solutions, emulsions, syrups, or elixirs, or may be presented dry in tablet form or a product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as
35 suspending agents, emulsifying agents, non-aqueous vehicles (which may

include edible oils), or preservative.

To prepare a vaccine, the LC3 peptide can be isolated, lyophilized and stabilized. The LC3 peptide may then be adjusted to an appropriate concentration, optionally combined with a suitable vaccine adjuvant, and packaged for use. Suitable adjuvants include but are not limited to surfactants, *e.g.*, hexadecylamine, octadecylamine, lysolecithin, dimethyldioctadecylammonium bromide, N,N-dioctadecyl-N'-N-bis(2-hydroxyethyl-propane di-amine), methoxyhexadecyl-glycerol, and pluronic polyols; polyanions, *e.g.*, pyran, dextran sulfate, poly IC, polyacrylic acid, carbopol; peptides, *e.g.*, muramyl dipeptide, MPL, aimethylglycine, tuftsin, oil emulsions, alum, and mixtures thereof. Other potential adjuvants include the B peptide subunits of *E. coli* heat labile toxin or cholera toxin. In one example, an active subunit of cholera CTA-a-DD is used (Mowat, 2001; Eriksson, 2003; Lycke, 2001). Other potential adjuvants are known in the art. Finally, the immunogenic product may be incorporated into liposomes for use in a vaccine formulation, or may be conjugated to proteins such as keyhole limpet hemocyanin (KLH) or human serum albumin (HSA) or other polymers.

The application of LC3 peptides for vaccination of a mammal against amebic colonization offers advantages over other vaccine candidates. Prevention of colonization or infection by inoculation not only reduces the incidence of the immediate symptoms, but also eliminates sequelae.

The active ingredient or mixture of active ingredients, in the vaccine composition is formulated conventionally using methods well known for formulation of protein or peptide vaccines. Vaccine compositions may include an immunostimulant or adjuvant such as complete or incomplete Freund's adjuvant, aluminum hydroxide, liposomes, beads such as latex or gold beads, ISCOMs, and the like. Liposomes are pharmaceutical compositions in which the active protein is contained either dispersed or variously present in corpuscles consisting of aqueous concentric layers adherent to lipidic layers. The active protein is preferably present in the aqueous layer and in the lipidic layer, inside or outside, or, in any event, in the non-homogeneous system generally known as a liposomic suspension. The hydrophobic layer, or lipidic layer, generally, but not exclusively, comprises phospholipids such as lecithin and sphingomyelin, steroids such as cholesterol, more or less ionic surface active substances such as dicetylphosphate, stearylamine or phosphatidic acid, and/or other materials of a

hydrophobic nature.

The vaccine compositions preferably contain (1) an effective amount of the active ingredient, that is, the peptide or peptides, together with (2) a suitable amount of a carrier vehicle, and, if desired, (3) preservatives, buffers, and the like.

The vaccines are administered as is generally understood in the art. With suitable formulation, peptide vaccines may be administered across the mucus membrane using penetrants such as bile salts or fusidic acids in combination, usually, with a surfactant. Transcutaneous administration of peptides is also known. Oral formulations can also be used. Dosage levels depend on the mode of administration, the nature of the subject, and the nature of carrier/adjuvant formulation. Preferably, an effective amount of the protein or peptide is between about 0.01 $\mu\text{g/kg}$ -1 mg/kg body weight. In general, multiple administrations of the vaccine in a standard immunization protocol are used, as is standard in the art.

LC3-specific Antibodies

The terms "antibody" and "antibodies" encompass intact molecules as well as fragments thereof that are capable of binding to LC3 subunit peptide. Antibodies can be polyclonal antibodies, monoclonal antibodies, humanized or chimeric antibodies, single chain Fv antibody fragments, Fab fragments, and F(ab)₂ fragments. Polyclonal antibodies are heterogeneous populations of antibody molecules that are specific for a particular antigen, while monoclonal antibodies are homogeneous populations of antibodies to a particular epitope contained within an antigen.

An antibody can be of any immunoglobulin (Ig) class, including IgM, IgA, IgD, IgE, and IgG, and any subclass thereof. Antibodies of the IgA class are particularly useful because one antibody molecule can cross-link another of LC3 subunit peptide, and are mucosal antibodies. Immune complexes containing Ig molecules that are cross-linked (*e.g.*, cross-linked IgG) and are thus multivalent also are capable of cross-linking a plurality of LC3 subunit peptide molecules, and can be particularly useful.

As used herein, an "epitope" is a portion of an antigenic molecule to which an antibody binds. Antigens can present more than one epitope at the same time. For polypeptide antigens, an epitope typically is about four to six

amino acids in length. Two different immunoglobulins can have the same epitope specificity if they bind to the same epitope or set of epitopes.

Polyclonal antibodies are contained in the sera of immunized animals. Monoclonal antibodies can be prepared using, for example, standard hybridoma
5 technology. In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture as described in the art. A hybridoma producing monoclonal antibodies of the invention can be cultivated *in vitro* or *in vivo*.

10 Antibodies of the invention also can be isolated from, for example, the serum of an individual. Suitable methods for isolation include purification from mammalian serum using techniques that include, for example, chromatography.

Antibodies that bind to LC3 subunit peptides also can be produced by, for example, immunizing host animals (*e.g.*, rabbits, chickens, mice, guinea pigs, or rats) with LC3 subunit peptides. An LC3 peptide or a portion of an LC3
15 peptide can be produced recombinantly, by chemical synthesis, or by purification of the native protein, and then used to immunize animals by injection of the polypeptide. Adjuvants can be used to increase the immunological response, depending on the host species. Suitable adjuvants include Freund's adjuvant (complete and incomplete), mineral gels such as
20 aluminum hydroxide, surface-active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin (KLH), and dinitrophenol. Standard techniques can be used to isolate antibodies generated in response to the LC3 immunogen from the sera of the host animals.

Antibodies can be produced recombinantly. The amino acid sequence
25 (*e.g.*, the partial amino acid sequence) of an antibody provided herein can be determined by standard techniques, and a cDNA encoding the antibody or a portion of the antibody can be isolated from the serum of the subject (*e.g.*, the human patient or the immunized host animal) from which the antibody was originally isolated. The cDNA can be cloned into an expression vector using
30 standard techniques. The expression vector then can be transfected into an appropriate host cell (*e.g.*, a Chinese hamster ovary cell, a COS cell, or a hybridoma cell), and the antibody can be expressed and purified.

Antibody fragments that have specific binding affinity for LC3 subunit peptides and retain cross-linking function also can be generated by techniques
35 such as those disclosed above. Such antibody fragments include, but are not

limited to, F(ab')₂ fragments that can be produced by pepsin digestion of an antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed. Single chain Fv antibody fragments are formed by linking
5 the heavy and light chain fragments of the Fv region via an amino acid bridge (e.g., 15 to 18 amino acids), resulting in a single chain polypeptide. Single chain Fv antibody fragments can be produced through standard techniques, such as those disclosed in U.S. Patent No. 4,946,778. Such fragments can be rendered multivalent by, for example, biotinylation and cross-linking, thus generating
10 antibody fragments that can cross-link a plurality of LC3 subunit peptides.

The invention will now be illustrated by the following non-limiting Examples.

EXAMPLE 1

15 Presence Anti-lectin IgG and IgA in Sera

Patients cured of amebic liver abscess (ALA) have immunity to *Entamoeba dispar* intestinal infection. Children cured of amebic colitis exhibit immunity to *E. histolytica* infections, associated with the presence of anti-lectin IgA antibodies. (Haque, 2002). The inventors studied the epitope specificity of
20 anti-lectin sera IgG and IgA antibodies in subjects cured of ALA or having asymptomatic intestinal infection. The results were correlated with inhibition of galactose-specific amebic adherence with epitope-specific mouse monoclonal antibodies (MoAb). Samples were obtained from 75 individuals: 42 ALA patients, 11 subjects with asymptomatic *E. histolytica* intestinal infection, and 22
25 uninfected controls. Reactivity was determined against seven overlapping fragments of LC3, a cysteine rich portion (AA758-1134) of the galactose-inhibitable lectin heavy subunit (Figure 1). All asymptomatically infected individuals and 19 of 42 ALA cases were positive by stool culture and fecal PCR for *E. histolytica* infection, while controls and the remaining ALA patients were
30 PCR and culture negative. All ALA patients and asymptomatic *E. histolytica* infections were positive for serum anti-LCX3 (full length) IgG antibodies. Sera from 32 of 40 ALA patients and 10 of 11 asymptomatic *E. histolytica* infections were also positive for anti-LC3 (full length protein) IgA antibodies. Serum anti-LC3 IgG and IgA antibodies exclusively recognized the third (868-944) and the
35 seventh (1114-1134) LC3 epitopes. A sixth epitope (1070-1114) was identified

by anti-LC3 IgG antibodies, but not by IgA antibodies. Mouse anti-LC3 IgA MoAbs recognized five out of seven LC3 fragments. The majority of the murine MoAbs recognized the first LC3 fragment (758-826), but was not recognized by human immune sera. Interestingly, adherence of *E. histolytica in vitro* to CHO cells was inhibited by a MoAb to the third fragment (61%), but not by a MoAb to the sixth fragment (28%). In conclusion, the third fragment of LC3 induces a humoral immune response (IgA and IgG) in humans with ALA or asymptomatic infection without variation among individuals. MoAb against this fragment inhibits the *in vitro* lectin-mediated adherence of *E. histolytica* to mammalian cells, suggesting this fragment might be a successful vaccine candidate for inducing mucosal immunity to *E. histolytica* infection.

EXAMPLE 2

Colonization of the gut by the enteric protozoan, *E. histolytica*, is associated with adherence to the carbohydrate-rich mucin layer covering the colonic mucosa (Chadee, 1987; Chadee, 1988), which forms a non-immune barrier to parasitic invasion. In general, secretory IgA antibodies are thought to contribute to mucosal defense via immune exclusion. IgA antibodies prevent contact of enteric pathogens with the intestinal epithelial surface due to their agglutination, entrapment within immune complexes, and clearance within the mucous blanket (McGhee, 1992).

Adherence of *E. histolytica* to colonic mucins and epithelial cells is mediated by the parasite's galactose-inhibitable surface lectin (Chadee, 1987; Petri, 1987). The carbohydrate-binding domain of the lectin's 170 kDa heavy subunit (Petri, 1990; Ravdin, 1981) is localized between amino acids 895 to 998 (Dodson, 1997; Mann, 1993; Pillai, 1999). Murine IgG monoclonal antibodies to the 170 kDa lectin subunit (Petri, 1987) completely abrogate the galactose-specific adherence of *E. histolytica* trophozoites to colonic mucins *in vitro* (Chadee, 1987; Chadee, 1988), indicating that intestinal anti-lectin IgA antibodies could have an important role in mucosal immunity to *E. histolytica*. There is mounting evidence from epidemiologic studies that intestinal anti-lectin IgA antibodies mediate immunity to intestinal infection by *E. histolytica* (Haque, 2001; Haque, 2002) and *E. dispar* trophozoites (Ravdin, 2003). The latter is a closely related but distinct species (Diamond, 1978) that is morphologically identical to *E. histolytica* and possess a functional galactose-binding lectin with

greater than 85% amino acid sequence homology to that of *E. histolytica*. The *E. dispar* lectin includes the complete carbohydrate-binding domain (Pillai, 1997). *E. dispar* induces an intestinal but not a humoral anti-lectin IgA antibody response (Ravdin, 2003).

5 A recombinant cysteine-rich fusion protein that includes amino acids 758-1134 of the lectin's 170 kDa subunit, designated LC3 (Soong, 1995), is recognized by adherence-inhibitory IgG monoclonal antibodies and includes the lectin's galactose-binding site (Dodson, 1997; Mann, 1993; Pillai, 1999). The LC3 protein is highly antigenic and immunogenic; purified LC3 protein has a
10 70% vaccine efficacy in the gerbil model of amebic liver abscess (Soong, 1995). Oral immunization of BALB/c mice with the LC3 protein with cholera toxin as adjuvant induces an adherence-inhibitory intestinal anti-LC3 IgA antibody response (Beving, 1996). Anti-LC3 IgA and IgG antibodies are present in the sera of over 90% of patients with invasive amebiasis (colitis and ALA) and in
15 the majority of subjects with asymptomatic *E. histolytica* intestinal infection (Abd-Alla, 2002; Ravdin, 2003). In several studies that encompassed large numbers of patients with amebic colitis or liver abscess, a mucosal IgA immune response to the recombinant LC3 antigen was detected (Abou-El-Magd, 1996; Ravdin, 2003).

20 The present inventors identified the specific LC3 epitopes recognized by IgA antibodies associated with the putatively protective mucosal immune response that occurs following cure of ALA (Ravdin, 2003). The IgA antibody epitopes were identified by use of overlapping recombinant LC3 protein fragments, utilizing serum IgG antibodies for comparison. The findings were
25 confirmed by studies with pooled intestinal IgA antibodies. The inventors produced IgA monoclonal antibodies to the LC3 protein for use as specific probes to correlate epitope-recognition with inhibition of amebic galactose-specific adherence. To further define the putative protective LC3 epitopes, overlapping peptides were prepared utilizing amino acid sequences of the
30 reactive LC3 epitopes and screened for recognition using IgA antibodies from pooled human sera and feces.

SUBJECTS AND METHODS

Subjects

35 Sera and stool samples were obtained from control subjects without

amebic infection, seropositive subjects with *E. histolytica* asymptomatic infection, patients recently (0-3 months) cured of ALA with or without a sustained *E. histolytica* intestinal infection, and ALA patients one year after cure who remained uninfected in a highly endemic area in Durban, South Africa.

5 Luminal amebicidal agents such as diloxanide furoate or paromomycin are unavailable in South Africa; therefore, ALA patients may remain infected for months to years after treatment (Ravdin, 2003). The presence of *E. histolytica* infection was determined by ribosomal DNA amplification using PCR and/or culture of stool with zymodeme analysis.

10

Detection of Amebic Infection by Ribosomal DNA PCR

Stool samples were stored at -70°C and the extracted DNA was stored at -20°C in a fecal DNA bank. The QIAamp DNA Stool Mini Kit (Quiagen, Haldin, Germany) was used to extract DNA human feces according to the
15 manufacture's protocol. 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 added in another area. PCR was run in a third area and the analysis and storage of the amplified PCR materials (materials, glass wear and equipments) occurred in the remaining area.

20 PCR was performed using dNTPs (Amersham Pharmacia Biotech, Catalog # 27-2035-01) by mixing 100 μ l of each nucleotide (G, C, T, and A; each nucleotide was supplied as a 100 mM solution in H₂O at pH 7.5) to 5 ml of 10x PCR Buffer and 4.6 ml H₂O (final concentration of each nucleotide was 10 μ l/ml = 5.8 μ g/ml for A, 5.6 μ g/ml for C, 5.9 μ g/ml for G, and 5.7 μ g/ml for T),
25 divided into 1 ml aliquots and stored at -20°C. The Taq Polymerase (Amersham Pharmacia Biotech, Catalog # 270799) was diluted as 1:20 immediately before use. The *E. histolytica* sense primer (5'- GTA CAA AAT GGC CAA TTC ATT CAA CG - 3' (SEQ ID NO:1)), *E. dispar* sense primer (5' - GTA CAA AGT GGC CAA TTT ATG TAA GCA - 3' (SEQ ID NO:2)) and *E.*
30 *histolytica*/*E. dispar* anti-sense primer (5' - GAA TTG ATT TTA CTC AAC TCT AGA G - 3' (SEQ ID NO:3)) (Blessman, 2002) were prepared as 10 Pmol/ μ l. Bovine Serum Albumin (BSA) (Pierce, 200 mg/ml, Catalog # 23210) was diluted with equal volume of H₂O (500 μ l BSA + 500 μ l H₂O) and kept at 4°C. The DNA to be tested (5 μ l) was added to the 95 μ l of PCR mixture to
35 complete 100 μ l. Each DNA sample was tested twice, once using *E. histolytica*

sense and once using *E. dispar* sense.

The conventional PCR machine thermocycling conditions were one cycle of 2 minutes at 95°C followed by 35 cycles of 1 minute at 94°C, 1 minute at 56°C, and 30 seconds at 72°C. The last single cycle occurred for 3 minutes at 72°C.

Specific detection of amplified DNA was achieved by gel electrophoresis. Digested DNA was separated on a 2% agarose gel containing ethidium bromide.

Expression of recombinant LC3 fragments in *E. coli*

The DNA encoding LC3 (bp 2273-3397 of the lectin-heavy subunit gene) (Soong, 1995; Tannish, 1991) was subject to restriction enzyme digestion (Figure 1) and the DNA fragments ligated inframe into pREST expression vectors. Transformed bacteria were grown and the fusion proteins expressed as detailed previously (Soong, 1995). Expression of each protein was verified by immunoblotting with a T7 tag IgG monoclonal antibody, which binds to the fusion leader sequence.

Generation of IgA monoclonal antibodies to the LC3-encoded protein fragments

BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, Maine) that maintains pathogen-free animal colonies. The mice were maintained in micro-isolation cages, free from Sendai virus and other pathogenic microorganisms.

IgA monoclonal antibodies were produced via a mucosal immunization protocol. BALB/c mice were immunized intra-Peyers patch twice with 200 µg of LC3 protein and boosted intravenously with 2 µg epinephrine IP and 50 µg of LC3 protein the following day. Three days later, the mice were scarified and their spleen cells hybridized to SP2/0 myeloma cells.

ELISA was utilized to identify hybridoma clones that secreted IgA monoclonal antibodies to the LC3-encoded protein (Nedrud, 1987). Nunc-Immune plates were coated overnight with LC3 protein at 4°C at pH 9.6. The plates were blocked with 1% BSA in phosphate-buffered saline solution (PBS). Tissue culture supernatants from each fusion were incubated for one hour at room temperature or over-night at 4°C. Following washing with PBS containing 1% BSA and 0.5% Tween 20, alkaline phosphatase conjugated goat anti-mouse

IgA antibodies were added as 100 µl/well at a concentration of 1 to 1000 in PBS-Tween with 1% BSA. The enzymatic reaction was developed with 1 mg/ml of p-nitrophenol phosphate substrate and the optical density (OD) was determined at a wavelength of 410 nm. An OD reading of 0.05 above the control well without LC3 present was considered positive. The isotype specificity of the anti-mouse IgA conjugate was confirmed with IgA, IgG, and IgM myeloma proteins. The isotype of the murine antibody was also confirmed using an Iso-strip³ mouse monoclonal antibody isotyping kit.

10 Epitope mapping of LC3 fragments recognized by human serum IgA and IgG serum antibodies

ELISA was performed as described previously (Abd-Alla, 2000). LC3 protein fragments were purified as described (Soong, 1995). Briefly, 96-well microtiter flat-bottomed polystyrene ELISA plates were coated with individual LC3 protein fragments (0.4 Tg/well) and the non-reactive sites were blocked with 1% BSA. Serum samples were studied at a 1:100 dilution for IgA and 1:250 for IgG, all in PBS-Tween - 1% BSA and incubated for 2 hours at room temperature. Alkaline phosphatase-conjugated goat anti-human IgA antibodies (ICN Biomedicals (Costa Mesa, California) or anti-human IgG (SIGMA, St. Louis, MO) were diluted (at 1:2500 for IgA and 1:5000 for IgG) in PBS-Tween - 1% BSA for incubation in 100 µl well for 2 hours at room temperature. Developing, reading the plates and correction of nonspecific background binding were performed as described (Ravdin, 1990).

25 Epitope mapping of LC3 fragments recognized by murine IgA monoclonal antibodies

Transformed bacteria (Soong, 1995) were pelleted, lysed in SDS, and loaded into 10% Laemmli polyacrylamide gels. After electrophoresis, the proteins were transferred to nitrocellulose papers for immunoblotting with anti-LC3 IgA monoclonal antibodies. Horseradish peroxidase-conjugated anti-mouse IgA (1:1000 dilution) was utilized as a secondary antibody and 4-chloro-1 naphthol as substrate for staining of the bound secondary antibody.

Effect of monoclonal antibodies on amebic *in vitro* adherence to CHO cells

35 *E. histolytica* trophozoites, strain HMI:IMSS were maintained in axenic

culture utilizing TYI-S-33 culture medium, as described by Diamond (Diamond, 1993), and harvested as described previously (Ravdin, 1981). CHO cells obtained from the ATCC were grown in F-12 medium (GIBCO) supplemented with 10% fetal bovine sera (GIBCO), penicillin (100 µg/ml) and streptomycin (100 µg/ml) as described previously.

Adherence studies were performed using a rosetting assay (Ravdin, 1981). Amebae (1×10^5 /ml) were incubated in hybridoma supernatant (dilution) at 4°C, control amebae were incubated with RPMI + 10%FBS, or tissue culture supernatant-containing anti-Sendai virus IgA monoclonal antibody. After extensive washing, trophozoites (1×10^4) and CHO cells (2×10^5) were suspended in 1 ml of M199S centrifuged at 250 x g and incubated for 2 hours at 4°C. After incubation, 0.8 ml of supernatant was removed and the pellet suspended. The percentage of amoebae that formed rosettes with CHO cells (three or more adherent cells) was determined in a hemocytometer chamber.

15

Synthesis and purification of peptides based on the amino acid sequences of the LC3 protein epitopes

Ten overlapping peptides were prepared from the amino acids sequence of epitope number 3 (between AA 868 and 944). Two more overlapping peptides were synthesized from sequences between amino acids 1114 and 1150 (epitope # 7).

Peptides were synthesized using Perkin Elmer Pioneer Peptide synthesizer, by solid-phase Fmoc (fluorenylmethoxycarbonyl) chemistry. Peptides were cleaved from the resin and de-protected using Reagent R, and then lyophilized. Lyophilized crude peptides were purified by preparative reverse-phase HPLC (Beckman 126) on a C4 column by VYDAC. Solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in ACN (Acetonitrile) on a gradient of 0-60% B in 30 minutes. Purity and quality control of the peptides were done by an analytical HPLC, HP1090 on a C18 (VYDAC) column using the same gradient and by Mass spectrometry on a HP MALDI_TOF (Atherton, 1978; Cleland, 1964).

30

Peptide recognition by human IgA and IgG antibodies

Pooled human sera and human feces 1.0 gm feces in 1.0 µl PMSF (2 mM) and 2.0 ml PBS-Tween containing 1.0% bovine serum albumin obtained

35

from ALA patients, asymptomatic *E. histolytica* infection and controls were used in ELISA to determine recognition of the reactive peptides. ELISA was performed in an identical method as assayed for LC3 fragments except equal volumes of sera or prepared feces from the study subjects were mixed and added in the previously mentioned concentration to wells coated with each peptide. The rest of the ELISA steps were identical to that performed with the protein fragments.

Statistics

Results were expressed as the mean, (+3 SD, of percent positive and percent negative). The Z test (converted to P value) and unpaired student t-test was used to determine the significance of difference (Sox 1986).

- Z test.

$$Z = (P1 - P2) \div \sqrt{PQ (1/N1 - 1/N2)}$$

P1 = proportion of positives in group one, P2 = proportion of positives in group two, P = pooled proportional estimate = $(X1 + X2) \div (N1 + N2)$, X1 = number of positives in group one, X2 = number of positives in group two, N1 = total number of group one, N2 = total number of group two, Q = (1 - P).

In the current study, the two sample z test is used for percents. The null hypothesis says that the percentages of positive test in the two groups are the same (difference in percentages = 0.0%). The alternative says that percentages of positive test in the two groups are different. Using type one error (alpha) equal 0.05:

- $Z > 1.645$ = significant changes.

- $Z < 1.645$ = insignificant changes.

RESULTS

Production of LC3 recombinant protein fragments

As illustrated in Figure 1, the LC3 protein encompasses amino acid 758 to 1134 of the lectin heavy subunit. LC3 DNA was cleaved by restriction enzyme digestion into two fragments (A and D) encoding proteins of: AA 758 – 944 and 944 – 1134. Fragment A was further digested into fragments B encoding (AA 758 – 868) and C (AA 758-826) (Figure 1). Fragment D was further digested into fragments E, F, and G encoding proteins of AA 944-1114, 944-1070, and 944-987, respectively. Recombinant proteins were produced

from each of these overlapping DNA fragments, the pattern of their recognition by IgA antibodies allowed identification of seven distinct LC3 epitopes.

Epitope mapping of LC3 fragments by human anti-LC3 IgA and IgG antibodies

5 The serologic response to purified LC3 protein was determined by ELISA for each of the study groups. Five different groups of amebiasis subjects were studied to determine if there were differences in IgA epitope specificity: (1) seropositive asymptomatic subjects with *E. histolytica* infection, (2) subjects cured of ALA with persistent asymptomatic intestinal infection or having cleared
10 the infection, and (3) ALA subjects who were infection free one year after cure. As expected, subjects recently cured of ALA with or without a current infection had higher OD readings for serum anti-LC3 IgA and IgG antibodies ($p < 0.05$ compared to controls, and ALA subjects one year after cure, Figures 2A and 2B). Seropositive subjects with asymptomatic infection had levels of anti-LC3
15 IgA and IgG antibodies comparable to that of the recently cured ALA subjects (Figures 2A and 2B). Serum anti-LC3 IgG antibody ELISA OD values (at equal dilutions) were higher than those for IgA antibodies ($p < 0.05$). In the epitope mapping studies, nonpurified recombinant proteins present in cell supernate were utilized as antigen in the ELISA. Therefore, nonpurified LC3 recombinant
20 protein present in *E. coli* supernate served as the positive control under identical experimental conditions as the recombinant LC3 fragments. Under these experimental conditions, ELISA for serum anti-LC3 IgA antibodies were reactive in 56.3% (one year after cure of ALA) to 90.1% (asymptomatic infected adults) of amebiasis subjects, all of whom were previously found to be IgA
25 seropositive to the highly purified LC3 protein (Table 1).

Table 1. Recognition of the *E. histolytica* LC3 recombinant protein fragments by serum IgA antibodies.

LC3 protein fragment	Seronegative & PCR (-) controls (n=22)	Asymptomatic Seropositive ^a & PCR (+) (n=11)	ALA Seropositive who are PCR (+) (n=19)	ALA Seropositive who are PCR (-) (n=23)	ALA Seropositive culture (-) after 1 year (n=16)
Nonpurified LC3	0	10 (90.1%)	16 (84.2%)	16 (69.6%)	9 (56.3%)
A (758-944)	0	6 (54.5%) ^a	15 (78.9%) ^b	14 (60.8%) ^a	8 (50%) ^b

B (758-868)	0	0	1 (5.3%)	0	0
C (758-826)	0	0	0	0	0
D (944-1134)	0	6 (54.5%) ^b	10 (52.6%) ^c	10 (43.4%) ^b	5 (31.5%) ^c
E (944-1114)	0	1 (9.1%)	3 (15.8%)	1 (4.3%)	0
F (944-1070)	0	1 (9.1%)	0	0	1 (6.3%)
G (944-987)	0	0	0	0	0

a- Seronegative or seropositive is defined by ELISA to purified LC3 protein, nonpurified LC3 protein serves as a positive control for nonpurified LC3 protein fragments.

5 b- $P < .05$ for recognizing LC3 fragment A compared to LC3 fragments B, C, E, F, and G.

c- $P < .05$ for recognizing LC3 fragment D compared to LC3 fragments B, C, E, F, and G.

10 Serum IgA antibodies obtained from all four amebiasis study groups recognized only LC3 fragments A (AA 758-944) and D (AA 944-1134), Table 1, ($p < 0.05$ compared to all other fragments and sera from uninfected seronegative controls). ELISA for anti-LC3 serum IgG antibodies demonstrated a higher level of reactivity than IgA to the nonpurified LC3 in *E. coli* supernate (81% to
15 100%, Table 2), concordant with the higher OD readings for serum IgG antibodies to the purified LC3 protein (Figure 2B). Serum IgG antibodies from three of the four amebiasis subgroups (asymptotically infected, ALA infected, ALA uninfected) recognized fragments A (AA 758-944), D (AA 944-1134) and E (AA 944-1114) ($p < 0.05$, Table 2). However, IgG antibody recognition of
20 fragment E was absent in sera from uninfected ALA subjects only one year after cure (Table 2).

Table 2. Recognition of the *E. histolytica* LC3 protein fragments by serum IgG antibodies

25

LC3 protein fragment	Seronegative & PCR (-) controls (n=22)	Asymptomatic Seropositive ^a & PCR (+) (n=11)	ALA Seropositive who are PCR (+) (n=19)	ALA Seropositive who are PCR (-) (n=23)	ALA Seropositive culture (-) after 1 year (n=16)
Nonpurified LC3	0	11 (100%)	18 (94.7%)	22 (95.7%)	13 (81.3%)
A (758-944)	0	11 (100%) ^b	16 (84.2%) ^b	19 (82.6%) ^b	10 (62.5%) ^b

B (758-868)	0	2 (18.2%)	0	0	0
C (758-826)	0	1 (9.1%)	0	0	0
D (944-1134)	0	11 (100%) ^c	15 (78.9%) ^c	19 (82.6%) ^c	10(62.5%) ^c
E (944-1114)	0	9 (81.2%) ^d	14 (73.7%) ^d	15 (65.2%) ^d	1 (6.3%) ^e
F (944-1070)	1(4.5%)	0	0	0	0
G (944-987)	0	0	0	0	1 (6.3%)

a - Seronegative or seropositive is defined by ELISA to purified LC3 protein, nonpurified LC3 protein serves as a positive control for nonpurified LC3 protein fragments.

b - $P < .05$ for recognizing LC3 fragment A compared to LC3 fragments B, C, F, and G.

c - $P < .05$ for recognizing LC3 fragment D compared to LC3 fragments B, C, F, and G.

d - $P < .05$ for recognizing LC3 fragment E compared to LC3 fragments B, C, F, and G.

e - $P < .05$ for recognizing fragment E compared to other ALA subjects and asymptomatic seropositive controls.

Based on the recognition pattern for the LC3 protein fragments, the inventors determined that human serum anti-LC3 IgA antibodies exclusively recognized LC3 epitopes three (AA 868-944) and seven (AA 1114-1134) regardless of study group (Figure 3). Anti-LC3 IgG antibodies also recognized epitope six (AA 1070-1114), but as stated above this recognition was lost 12 months after cure of ALA (Figure 3).

Characterization of murine anti-LC3 IgA monoclonal antibodies.

The inventors screened 1300 hybridoma clones by ELISA for anti-LC3 IgA antibodies and found 85 positive secretors. Of the 85, by limiting dilution, the inventors found 14 that were stable as single clones. The inventors confirmed by immunoblotting whether the 14 hybridoma clones produced IgA monoclonal antibodies to LC3 protein (Table 3). All but 2 of the 14 monoclonal antibodies, numbers 244 and 728, recognized native *E. histolytica* lectin by ELISA. IgA monoclonal antibodies to Sendai virus served as a negative control in all experiments.

Table 3. Epitope Specificity of fourteen anti-LC3 IgA murine monoclonal antibodies with comparison to that of human serum IgA and IgG anti-lectin

antibodies

LC3-epitope (Amino Acids)	IgA Monoclonal antibodies (Clone #)	Human	
		IgA	IgG
1. (758-826)	38, 41, 193, 244, 606, 728, 737, 854	(-)	(-)
2. (826-868)	(-)	(-)	(-)
3. (868-944)	875	(+)	(+)
4. (944-987)	580, 1152	(-)	(-)
5. (987-1070)	676, 1059	(-)	(-)
6. (1070-1114)	867	(-)	(+) ^a
7. (1114-1150)	(-)	(+)	(+)

a = Epitope recognition lost one year after cure of ALA

5 Anti-LC3 IgA monoclonal antibodies were further characterized by epitope mapping as shown in Table 3. The seven LC3 protein fragments (A-G) were immunoblotted with all 14 of the IgA monoclonal antibodies; the LC3 protein and a LC1 fusion protein (AA 1-346) were used as positive and negative controls respectively. Eight monoclonal antibodies (numbers 38, 41, 193, 244,
10 606, 728, 737, and 854) recognized fragments A, B, and C. Clone 875 reacted only with fragment A, indicating it recognized epitope 3 (AA 868-944). The inventors determined by similar analysis the epitope specificity of each anti-LC3 IgA monoclonal antibody (summarized in Table 3 and illustrated in Figure 3). None of the IgA monoclonal antibodies were found to recognize the second or
15 seventh LC3 epitopes (AA 826-868 and 1114-1134) (Figure 2). Interestingly, the overwhelming majority (13 of 14) of the murine IgA monoclonal antibodies recognized LC3 epitopes not recognized by human anti-LC3 IgA antibodies (Table 3 and Figure 3).

20 Effects of anti-LC3 IgA monoclonal antibodies on amebic adherence to CHO cells.

Except for clone 1152 (epitope 4) which demonstrated no inhibition, all of the anti-LC3 IgA monoclonal antibodies inhibited amebic galactose-specific adherence to CHO cells, regardless of epitope specificity, by a range of 25% to

87%, $P < 0.01$, compared to a control IgA monoclonal antibody (Table 4).

Monoclonal antibody 580, which also recognized epitope 4, inhibited amebic adherence by 72% ($p < .01$, Table 4).

5 Table 4. Inhibition of amebic adherence to CHO cells by anti-LC3 IgA murine monoclonal antibodies

IgA-producing Hybridoma Supernate (LC3 epitope)	Inhibition of Adherence ^{a,b}	IgA-producing Hybridoma Supernate (LC3 epitope)	Inhibition of Adherence ^{a,b}
38 (1)	73%	875 (3)	61%
41 (1)	25%	580 (4)	72%
193 (1)	70%	1152 (4)	3%
244 (1)	29%	676 (5)	79%
606 (1)	73%	1059 (5)	68%
728 (1)	36%	867 (6)	28%
737 (1)	87%	Control anti-Sendia virus IgA	1%
854 (1)	76%		

^a Percent inhibition of adherence defined as comparison to control test medium without antibodies present.

10 ^b Cell supernates from all anti-LC3 IgA MoAb inhibited amebic adherence except MoAb 1152, $P < 0.01$ compared to control anti-Sendia virus IgA.

Fine mapping of LC3 epitopes by use of synthetic peptides.

Pooled human sera and feces were studied for reactivity with twelve overlapping peptides to further define the LC3 epitopes recognized by human
 15 IgA antibodies. Ten peptides were prepared from the amino acid sequence of epitope three and two peptides were synthesized based upon the sequence of epitope seven. Fecal anti-LC3 IgA antibodies from ALA patients with current *E. histolytica* infection recognized the same four peptides (Figure 4), three of which were also recognized by serum IgA antibodies. Fecal and serum IgA antibodies
 20 from asymptomatic seropositive infected subjects recognized three of the four peptides (numbers 2, 9, and 12; Figure 4). The amino acid sequences of the reactive peptide segments as follows:

Peptide # 2, (AA 891-903) (TGT ACA TAC GAA ATA ACA ACA
 25 AGA GAA TGT AAA ACA TGT (SEQ ID NO: 4), CTYEITTRECKTC (SEQ ID NO:5));

Peptide # 9, (AA 918-936) (TGT GCA GAA GAG ACT AAG AAT GGA GGA GTT CCA TTC AAA TGT AAG AAT AAC AAT TGC (SEQ ID NO:6), CAETKNGGVVPFKCKNNNC (SEQ ID NO:7);

5 Peptide # 11, (AA 1114-1138) (TGT GAT CAA ACA ACT GGA GAA ACT ATT TAC ACA AAG AAA ACA TGT ACT GTT TCA GAA GAA TTC CCA ACA ATC ACA (SEQ ID NO:8), CDQTTGETIYTKKTCTVSEEFPTIT (SEQ ID NO:9));

10 Peptide # 12, (AA 1128-1150) (TGT ACT GTT TCA GAA GAA TTC CCA ACA ATC ACA CCA AAT CAA GGA AGA TGT TTC TAT TGT CAA TGT TCA (SEQ ID NO:10), CTVSEEFPTITPNQGRFCFYCQCS (SEQ ID NO:11)).

IgA antibodies from pooled control sera or in feces from controls did not react with any of the peptides, the four reactive peptides were also recognized by serum anti-LC3 IgG antibodies pooled from the same study subjects (data not shown). Therefore, the LC3 epitopes recognized by human serum and intestinal IgA antibodies regardless of clinical status are AA 891-903 and AA 918-936 of epitope three and all of epitope seven (AA 1114-1150). LC3 (and epitope 7) ends at AA 1134, but Peptide # 12 continues on to AA 1150.

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DISCUSSION

Human infection with *E. histolytica* and *E. dispar* results in an intestinal IgA antibody response to the 170-kDa galactose-inhibitable lectin subunit (Haque, 2001; Haque, 2002; Ravdin, 2003). Anti-lectin IgA antibodies have been found in saliva and feces of patients with invasive amebiasis (colitis or ALA) and subjects asymptomatically infected with *E. histolytica* (Abou-El-Magd, 1996; Haque, 2002). After cure of ALA, secretory anti-lectin IgA antibodies can be recognized in stool for up to 36 months (Ravdin, 2003). The relationship between intestinal anti-amebic IgA antibodies and protection against parasitic infection has been recognized in children studied in Bangladesh.

30 Immunity was reported to relate to individuals possessing anti-CRD (LC3 AA 895 to 998) IgA antibodies in feces (Haque, 2001). Those with other anti-lectin IgA antibodies were reported not to be immune (Haque, 2002). However, these IgA antibodies were demonstrated to be present in children for a very short time (one month) (Haque, 2001; Haque, 2002). Following cure of ALA in Durban, South Africa, protective immunity to *E. dispar* infection persists for at least three

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years, ALA subjects demonstrated sustained secretion of high titer intestinal anti-lectin IgA antibodies for up to 36 months (Ravdin, 2003). However, despite the presence of high titer anti-amebic IgA antibodies in feces, in the absence of luminal amebicidal agents, a significant percentage of ALA subjects remain
5 infected with *E. histolytica*. This indicates that there is a difference between immune clearance of established infection versus immunity to acquisition of a new infection. Such differentiation requires genotyping to distinguish new from on-going infections and established from transient infections (Soong, 1995; Zaki, 2003). Genotyping has already succeeded in identifying different *E.*
10 *histolytica* isolates in families residing in hyperendemic areas in South Africa. In most cases, members of individual family groups were infected with the same genotype of *E. histolytica* or *E. dispar* and the genotype remained constant over time (Zaki, 2003).

By using seven overlapping fragments of the recombinant LC3 protein,
15 the inventors determined that serum IgA antibodies from asymptotically infected subjects, seropositive ALA subjects (with or without current *E. histolytica* infection), and uninfected ALA subjects one year after cure exclusively recognized LC3 epitopes 3 (AA 868-944) and 7 (AA 1114-1134). Serum anti-LC3 IgG antibodies from recently cured ALA subjects also
20 recognized epitope six (AA 1070-1114), but this reactivity was lost by one year. There was no difference in epitope recognition between ALA subjects with or without sustained intestinal infection. This suggests that immunity to new asymptomatic *Entamoeba* species infection as observed by Haque et al. (Haque, 2002) and Ravdin et al. (Ravdin, 2003) does not correlate with the ability to
25 clear an established infection. Unlike Haque et al. (Haque, 2002), the present inventors found no clinical or immunologic subgroup that demonstrates a unique lectin epitope recognition pattern. Although the inventors cannot rule out the presence of additional IgA lectin epitopes that were not identified due to the sensitivity of the assay, clearly, epitopes three and seven were immuno-
30 dominant. In fact, previous studies indicate that the titer of anti-lectin IgA in stool may be the strongest predictor of effective mucosal immunity in adults (Ravdin, 2003). Infection by *E. dispar* does induce an intestinal anti-lectin IgA response, but it is of low titer and short lived (Ravdin, 2003). Therefore, it is not surprising that despite multiple shared lectin epitopes (Petri, 1990), *E. dispar*
35 infection does not induce cross-species protection against *E. histolytica* (Ravdin,

2003).

The present inventors utilized murine anti-LC3 IgA monoclonal antibodies as epitope-specific probes to correlate *in vitro* adherence-inhibitory activity with human IgA epitope specificity. Of interest, immunization of
5 BALB/c mice raised antibodies mainly to LC3 epitopes that are not recognized by humans (epitopes 1, 4, and 5 with epitope 1 predominate). Only one of fourteen murine IgA antibodies recognized an epitope shared by human IgA antibodies (epitope 3). Clearly, vaccine studies using lectin-derived proteins in murine experimental models must be interpreted with caution due to clear
10 differences in MHC-restricted immune recognition of the lectin protein structure. It would seem unwise to jump directly from murine models to studies in humans without conducting vaccine studies in a more immunologically related model, such as primates.

Murine IgA monoclonal antibodies possessed adherence-inhibitory
15 activity against amebic native surface lectin regardless of which LC3 epitope the antibody recognized. Interestingly, no adherence-enhancing activity (Petri, 1990) was observed with any of the IgA monoclonal antibodies studied. Analogous to studies of murine IgG or IgM anti-lectin monoclonal antibodies (Petri, 1987), adherence inhibitory activity of these IgA antibodies did not
20 correlate with direct recognition of the carbohydrate binding domain (AA 895-998) (Dodson, 1997; Mann, 1993; Pillai, 1999), which is contained within epitope four and extends partially to epitopes three and five. Therefore, the ability of anti-lectin IgA antibodies to mediate immunity in the gut may relate to multiple factors important in forming immune complexes and preventing the
25 parasite from binding to colonic mucins or host cells.

The LC3 protein does not include the lectin's pseudo-repeat region (AA 436-624), to which Lotter et al. (Lotter, 1997) raised adherence-inhibitory antibodies. However, as the LC3 protein includes the parasite's carbohydrate binding domain (Dodson, 1997; Pillai, 1999), is sufficient to induce immunity to
30 ALA in gerbils (Soong, 1995) (as is a smaller 375 amino acid fragment of LC3 (Dodson, 1999)), the inventors chose this cysteine rich recombinant protein for further study. It is possible that IgA antibodies to the pseudo-repeat region may also be important in host mucosal immunity. However, in contrast to the inventors' study of anti-LC3 IgA antibodies, immunity to the pseudo-repeat
35 region was found to wane more rapidly over time (Dodson, 1999).

Peptide synthesis has been considered a productive tool for preparation of short protein segments with a limited number of amino acids. Overlapping sub-fragments from each epitope was engineered through peptide synthesis (Atherton, 1978; Fields, 1989) to better define the human IgA epitope specificity. Both serum IgA and IgG antibodies recognized four of thirteen synthetic peptides when studied in either a linear or cyclized form. Therefore, the complete epitope specificity of human anti-LC3 IgA antibodies obtained from Durban, South Africa is defined as AA 891-903, AA 918 to 936, and AA 1114 to 1150. Peptides such as these can be prepared in multiple forms (Fields, 1989) or attached to a polylysine backbone (Tam, 1988) to further enhance immunogenicity for use as a subunit vaccine.

In summary, based on previous epidemiologic studies (Haque, 2001; Ravdin, 2003) and the current findings, the inventors have defined epitopes of the *E. histolytica* galactose-inhibitable lectin that are protective in humans. Identification of the lectin epitopes by IgA antibody recognition of synthetic peptides provides an amebiasis subunit vaccine for prevention of amebic intestinal infection in humans.

EXAMPLE 3

Development of a Synthetic Intranasal Lectin-based Amebiasis Subunit Vaccine for study in Baboons

E. histolytica is the third leading parasitic cause of death worldwide. Recent field studies indicate that following cure of invasive amebiasis, intestinal IgA antibodies to the amebic galactose-inhibitable lectin provide immunity to new *E. histolytica* and *E. dispar* infections. The recombinant LC3 protein is a cysteine-rich protein (AA 758 to 1134) of the lectin heavy subunit that includes the carbohydrate-binding domain, is highly antigenic, and effective as a subunit vaccine in the gerbil model of amebic liver abscess. Using restriction enzyme digest generated overlapping LC3 gene products, ELISA of serum and fecal IgA antibodies from human subjects cured of ALA (with or without infection) and those asymptotically infected, the inventors found all that the IgA antibodies exclusively recognized two of seven LC3 epitopes: epitope 3 (AA 868-944), and epitope 7 (AA 1114-1134) from all subjects. By use of synthetic overlapping peptides the inventors fine mapped the putatively protective epitopes to four discrete peptides sequences:

AA 891-903: TGT ACA TAC GAA ATA ACA ACA AGA GAA TGT
AAA ACA TGT (SEQ ID NO: 4), CTYEITTRECKTC (SEQ ID NO:5)

AA 918-936: TGT GCA GAA GAG ACT AAG AAT GGA GGA GTT
CCA TTC AAA TGT AAG AAT AAC AAT TGC (SEQ ID NO:6),

5 CAETKNGGVFPKCKNNNC (SEQ ID NO:7)

AA 1114-1138: (TGT GAT CAA ACA ACT GGA GAA ACT ATT TAC
ACA AAG AAA ACA TGT ACT GTT TCA GAA GAA TTC CCA ACA ATC
ACA (SEQ ID NO:8), CDQTTGETIYTKKTCTVSEEFPTIT (SEQ ID NO:9)

AA 1128-1150: TGT ACT GTT TCA GAA GAA TTC CCA ACA ATC
10 ACA CCA AAT CAA GGA AGA TGT TTC TAT TGT CAA TGT TCA (SEQ ID
NO:10), CTVSEEFPTITPNQGRFCFYCQCS (SEQ ID NO:11)

Synthetic peptides produced to mimic these from epitopes were randomly
linked to a polylysine backbone (6 peptides per molecule) and administered to
15 baboons intranasally (400 µg) x 4 at seven-day intervals with cholera holotoxin
(20 µg) as adjuvant, the recombinant LC3 protein served as a positive control.
The inventors found that the intranasal lectin-based synthetic peptide vaccine
induced anti-peptide, anti-LC3, and anti-lectin (purified native protein) intestinal
IgA and serum IgG antibodies by day 28 of the vaccine protocol.

20 In contrast, the recombinant LC3 protein vaccine induced serum anti-
LC3 and anti-lectin IgA and IgG antibody responses, but did not elicit any
intestinal anti-lectin IgA antibody response. In addition, the LC3 vaccine-
elicited serum IgA and IgG antibodies did not recognize any of the four
putatively protective LC3 epitopes as defined by IgA antibody ELISA with
25 human serum and feces. In summary, the inventors developed an intranasal
synthetic peptide amebiasis subunit vaccine that elicited a mucosal IgA antibody
response in baboons that mimics that of humans cured of invasive amebiasis.

Briefly, nine seronegative and PCR-negative baboons were selected and
divided into three groups. The animals were challenged with peptides and
30 adjuvant (cholera toxin, CT) intranasally. They were immunized on Day 1, 7,
14, and 21 for a total of four intranasal immunizations.

Group 1: 3 baboons CT (20 µg in 100 µl) + saline (100 µl) in each
nostril (200 µl total) as a negative control;

Group 2: 3 baboons CT (20 µg in 100 µl) + 400 µg peptide mixture in
35 100 µl saline in each nostril (200 µl total); and

Group 3: 3 baboons CT (20 µg in 100 µl) + 200 µg LC3 (200µg) in 100 µl saline in each nostril (200 µl total) as a positive control.

The results are provided in Figures 5-13. Briefly, both the intranasal
5 recombinant LC3 vaccine and the synthetic peptide vaccine induced serum IgG
antibodies to the recombinant LC3 protein as determined by ELISA (Figure 5).
Importantly, both the recombinant LC3 protein and the synthetic peptide vaccine
induced serum IgG antibodies that recognized native Gal/GalNAC lectin from *E.*
histolytica trophozoites, which is present on the surface of the parasite (Figure
10 6).

Only the synthetic peptide vaccines induced serum IgG antibodies to the
four putatively protective LC3 epitopes, as determined by ELISA with purified
peptides (AA 891-903, 918-936, 1114-1138, and 1128-1150). The recombinant
LC3 vaccine induced IgG antibodies to itself (Figure 5) and the native lectin
15 (Figure 6), but not to the four putatively protective LC3 epitopes defined by
mapping with human IgA antibodies (Figure 7). Only the recombinant LC3
protein vaccine induced a serum anti-LC3 IgA antibody response; none was
observed with the synthetic peptide vaccine (Figure 8). As shown in Figure 8,
the recombinant LC3 vaccine but not the synthetic peptide vaccine induced
20 serum anti-lectin IgA antibody response (Figure 9). Consistent with the results
of LC3 recombinant vaccine induced serum IgG antibodies (Figure 7),
recombinant LC3 protein vaccine (Figures 8 and 9) did not elicit serum IgA
antibodies that recognize the four peptide epitopes; the synthetic peptide vaccine
elicited a weak serum anti-peptide IgA antibody response (Figure 10). As is
25 evident, only the synthetic peptide vaccine induced a fecal (intestinal) anti-LC3
IgA antibody response; the recombinant LC3 protein vaccine induced serum
anti-LC3 IgA antibodies (Figure 8), but not a fecal or mucosal anti-LC3 IgA
antibody response (Figure 11). Importantly, the synthetic peptide vaccine
induced intestinal IgA antibodies that recognized the amebic native
30 Gal/Gal/NAC lectin molecule present on the surface of the parasite; again, no
mucosal IgA antibody response was observed with the LC3 recombinant protein
vaccine (Figure 12). As expected, only the synthetic peptide vaccine induced
mucosal IgA antibodies to the four putatively protective LC3 peptide epitopes;
no response was observed with the LC3 protein vaccine (Figure 13).

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SUMMARY

Mucosal anti-lectin IgA antibodies provided effective immunity against *E. histolytica* and *E. dispar* infections. The frequency of *E. histolytica* and *E. dispar* infection in endemic areas is greater than previously reported, eliciting amnestic intestinal anti-lectin IgA antibody responses. Regardless of clinical phenotype, serum and intestinal IgA antibodies from subjects in Durban, S. Africa recognize four discrete LC3 epitopes. Based on the LC3 epitope amino acid sequence, an experimental synthetic peptide vaccine delivered intranasally with cholera toxin as adjuvant to baboons induced an intestinal anti-peptide, anti-LC3, and anti-lectin IgA antibody response. It is important to note that only the peptide vaccine, and not the recombinant LC3 vaccine, induces serum and intestinal IgA antibodies to the four putatively protective epitopes.

All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

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What is claimed is:

1. A vaccine comprising an *Entamoeba histolytica* LC3 subunit peptide, wherein the LC3 subunit peptide has the sequence represented by amino acids numbered 868-944 (LTSYKLNLPQSEEFMKEADKEAYCTYEITTRECKTCSLTETKEKVEEIDLCAEETKNGGVVPFKCKNNNCIIDPNFDC), amino acids numbered 891-903 (CTYEITTRECKTC (SEQ ID NO:5)), amino acids numbered 918-936 (CAEETKNGGVVPFKCKNNNC (SEQ ID NO:7)), amino acids numbered 1114-1134 (CDQTTGETIYTKKTCTVSEEF), amino acids numbered 1114-1138 (CDQTTGETIYTKKTCTVSEEFPTIT (SEQ ID NO:9)), or amino acids numbered 1128-1150 (CTVSEEFPTITPNQGRFCFYCQCS (SEQ ID NO:11)) wherein the vaccine induces mucosal anti-lectin IgA antibody production in a primate against *Entamoeba histolytica* or *E. dispar*.
2. The vaccine of claim 1, wherein the LC3 subunit peptide has the sequence represented by amino acids numbered 868-944.
3. The vaccine of claim 1, wherein the LC3 subunit peptide has the sequence represented by LC3 amino acids numbered 891-903.
4. The vaccine of claim 1, wherein the LC3 subunit peptide has the sequence represented by LC3 amino acids numbered 918-936.
5. The vaccine of claim 1, wherein the LC3 subunit peptide has the sequence represented by LC3 amino acids numbered 1114-1134.
6. The vaccine of claim 1, wherein the LC3 subunit peptide has the sequence represented by LC3 amino acids numbered 1114-1138.
7. The vaccine of claim 1, wherein the LC3 subunit peptide has the sequence represented by LC3 amino acids numbered 1128-1150.
8. The vaccine of claim 1, wherein the LC3 subunit peptide is in combination with a vehicle.

9. The vaccine of claim 1, wherein the LC3 subunit peptide is expressed from an isolated DNA sequence encoding the LC3 subunit peptide, wherein the DNA encodes an LC3 subunit peptide that comprises contiguous amino acid residues from residue 891 to residue 903 (TGT ACA TAC GAA ATA ACA ACA AGA GAA TGT AAA ACA TGT (SEQ ID NO: 4)), from residue 918 to residue 936 (TGT GCA GAA GAG ACT AAG AAT GGA GGA GTT CCA TTC AAA TGT AAG AAT AAC AAT TGC (SEQ ID NO:6)), from residue 1114 to residue 1138 (TGT GAT CAA ACA ACT GGA GAA ACT ATT TAC ACA AAG AAA ACA TGT ACT GTT TCA GAA GAA TTC CCA ACA ATC ACA (SEQ ID NO:8)), or from residue 1128 to residue 1150 (TGT ACT GTT TCA GAA GAA TTC CCA ACA ATC ACA CCA AAT CAA GGA AGA TGT TTC TAT TGT CAA TGT TCA (SEQ ID NO:10)).
10. The vaccine of claim 9, wherein the DNA encodes an LC3 subunit peptide that comprises contiguous amino acid residues from residue 891 to residue 903.
11. The vaccine of claim 9, wherein the DNA encodes an LC3 subunit peptide that comprises contiguous amino acid residues from residue 918 to residue 936.
12. The vaccine of claim 9, wherein the DNA encodes an LC3 subunit peptide that comprises contiguous amino acid residues from residue 1114 to 1138.
13. The vaccine of claim 9, wherein the DNA encodes an LC3 subunit peptide that comprises contiguous amino acid residues from residue 1128 to residue 1150.
14. The vaccine of claim 9, wherein the LC3 subunit peptide is a variant of wild-type LC3 subunit peptide in that the variant LC3 subunit peptide has a substitution at one or more amino acid residues and wherein the variant LC3 subunit peptide has at least 90% contiguous amino acid sequence identity to the amino acid sequence of a corresponding native LC3.
15. The vaccine of claim 14, wherein the substitution is a conservative substitution.
16. The vaccine of claim 1, which further comprises an immunological adjuvant.

17. The vaccine of claim 1, wherein at least one LC3 subunit peptide is conjugated or linked to a carrier molecule, wherein the carrier molecule is a polypeptide or a polysaccharide.
18. The vaccine of claim 17, wherein two or more different LC3 subunit peptides are conjugated or linked to the same carrier molecule.
19. The vaccine of claim 1, wherein the primate is human.
20. The LC3 subunit peptide vaccine of claim 1 for use in, wherein the LC3 subunit peptide vaccine induces mucosal anti-lectin IgA antibody production in a primate, and wherein the LC3 subunit peptide is in combination with a vehicle.
21. The vaccine of claim 20, wherein the vaccine is administered by subcutaneous or intramuscular injection.
22. The vaccine of claim 20 wherein the vaccine is administered by oral ingestion.
23. The vaccine of claim 20 wherein the vaccine is administered intranasally.
24. The vaccine according to claim 23 wherein the primate is human.
25. The used of the vaccine of claim 1 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of *Entamoeba histolytica* or *E. dispar* colonization or infection.