Journal of the Egyptian Society of Parasitology, Vol.43, No.3, December 2013
J. Egypt. Soc. Parasitol., 43(3), 2013: 723 – 735

NATURAL INFECTION OF BABOONS BY *ENTAMOEBA HISTOLYTICA* ELICITS ANTI- GAL-LECTIN HEAVY SUBUNIT IGA AND IGG ANTIBODIES WITH SHARED EPITOPE SPECIFICITY TO THAT OF HUMANS

By

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Abstract

Non-human primates, such as baboons (*Papio hamadryas anubis*), are natural hosts for *Entamoeba* species; infections can be asymptomatic or result in invasive lethal disease. It was sought to determine whether following natural infection by *Entamoeba. histolytica*, baboon anti-amebic antibodies recognized native Gal-lectin, a recombinant portion of the lectin heavy subunit (designated LC3) and specific heavy subunit epitopes; we compared the specificity of anti-amebic antibodies from baboons to that of humans following asymptomatic *E. histolytica* infection or cure of amebic liver abscess (ALA). Female baboons (n=54), aged one to three years of age and living in captivity were screened for infection by real time PCR. *E. histolytica* infection was found in 37 baboons and was associated with serum anti-LC3 IgG (73%) and anti-LC3 IgA (46%) or intestinal anti-Gal-Lectin IgA antibody responses (49%), $p<0.021$ for each compared to that observed with baboons having an *E. dispar* infection (n=10) or uninfected baboons (n=7). The ELISA OD reading for anti-LC3 or anti-lectin antibodies correlated strongly with the presence of a PCR CT value indicative of *E. histolytica* infection. In humans with asymptomatic *E. histolytica* infection or those recently cured of ALA, 63% and 57% had serum anti- LC3 IgA and 65% and 57% had serum anti-LC3 IgG antibodies respectively. Epitope- specific synthetic peptides were used as capture antigens in ELISA; for baboons that possessed anti-LC3 and anti-lectin antibodies, 74% had anti-peptide IgG or IgA antibodies, compared to 86% of asymptomatic humans and 92% of ALA subjects($P>0.05$).

Keywords: *Entamoeba histolytica;* baboons Papio; Gal-lectin; natural infection; mucosal immunity.

Introduction

Given the susceptibility of old world primates to *E. histolytica* infection, experimental infection and host immunity should be studied (Jackson *et al,* 1990; Pang *et al,* 1993; Verweij *et al,* 2003a). Mucosal and humoral immune responses and antibody specificity to the *E. histolytica* Gal-lectin, the function of which is essential for the parasite`s colonization of the gut and pathogenesis of disease (Petri *et al,* 1987, Chadee *et al,* 1987, Ravdin *et al,* 1981), has not been defined. The immune response to experimental *E histolytica* infection has been studied in mice, gerbils, rats, and guinea pigs, (Krupp 1974; Ghadirian and Meerovitch, 1978; 1980; Jain *et al,* 1980; Martinez *et al,* 1980; Sepulveda 1980; Perti and Ravdin 1990, Soong *et al,* 1995; Lotter *et al,* 2000; Ivory and Chadee 2007; Carrero *et al,* 2010; Cho *et al,* 2010; Guo *et al,* 2008, 2011; Becker *et al,* 2011). However, natural infection with *E. histolytica* does not occur in these experimental hosts. A high prevalence of *E. histolytica* infection (41% & 44% respectively) has been reported in captive non-human primates, as detected by PCR (Levecke et al., 2010, 2007). Wild-trapped non-human primates have a lower prevalence of *E. histolytica* (24%) compared to (64.4%) infection with helminthes (Munene *et al,* 1998).

Infection of humans with *E. histolytica* induces intestinal and serum anti-amebic antibodies that recognize native Gal-lectin (Ravdin *et al,* 1990, Abou Al-Maged *et al,* 1996) and a recombinant protein (designated LC3) that encompasses amino acids 758 to 1134 of the lectin`s heavy subunit (Soong *et al,* 1995). Specific IgG and IgA epitopes of the LC3 protein were determined by patterns of antibody recognition of overlapping recombinant LC3 protein fragments in ELISA, followed by fine mapping using the same strategy with synthetic peptides (Abd-Alla *et al,* 2004). Intranasal immunization of baboons with epitope-specific synthetic peptides with cholera toxin as adjuvant elicited serum IgG and intestinal IgA antibodies that recognized native lectin, purified recombinant LC3 protein and inhibited the in vitro galactose-specific adherence of *E. histolytica* trophozoites to Chinese hamster ovary cells (Abd-Alla *et al*, 2007).

The goals of the research reported herein were to determine the prevalence of asymptomatic *E. histolytica* infection in baboons living in captivity, whether such infections elicited serum and intestinal antibodies to the *E. histolytica* Gal-Lectin heavy subunit, and if baboons and humans exhibit shared heavy subunit epitope specificity. The outcome of such studies would be of significance in regard to the use of baboons as experimental hosts of *E. histolytica* for study of host immunity and development of an amebiasis subunit vaccine with high potential for application in humans.

Materials and Methods

Baboon Population: Baboons (*Papio hamadryas anubis*) of both genders and various ages were housed as large breeding groups within indoor and outdoor areas. When obtaining samples, baboons were single-housed in alumi-

num cages at the University of Oklahoma Health Sciences Center's (OUHSC) Comparative Medicine's facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures were approved by the OUHSC and University of Minnesota Institutional Animal Care and Use Committees. Baboons were fed a commercial monkey chow twice daily and given fruit, popcorn, peanuts, and other treats once a day.

The 54 female baboons, ages one to three years, utilized in this study were not previously included in other research projects and didn't exhibit any physical or behavioral abnormalities before enrollment. All of the baboons were living under the same conditions and managed by the same personnel. To obtain blood samples, baboons were sedated with Ketamine hydrochloride (approximately 10mg/kg/IM) and 10 ml was collected by venipuncture, placed into a serum separator tube on ice. After clotting for 2 hours, the samples were centrifuged for 20 minutes at high speed. Sera were stored at -80^{o}C and shipped on dry ice to the Ravdin laboratory. Stools were collected from each animal separately by placing a clean pad under each cage; fecal samples were aliquoted into a 5ml tube, frozen in -80 freezers and also sent on dry ice to the Ravdin laboratory.

Human Infection by *E. histolytica*: Subjects were randomly selected from a prospective longitudinal controlled field study performed in Durban, South Africa (Ravdin *et al,* 2003, Abd-Alla *et al,* 2006) that included a total of 954 controls and 100 index cases(recently cured of amebic liver abscess)(ALA) who were followed every three months for three years. Subjects for this study were randomly selected from the control group (n=40) when they had a documented asymptomatic *E. histolytica* infection, as determined by fecal culture and zymodeme (Ravdin *et al,* 2003) and from the index case cohort (n=35) immediately after being cured of amebic liver abscess. Sera from both groups were evaluated by ELISA for anti-LC3 and anti-peptide IgA and IgG antibodies as described below. Studies were performed concurrently with baboon serum ELISA.

Detection of *E. histolytica* and *E. dispar* infection of baboons by real time PCR: DNA was extracted from stool samples and stored at -20 C. The QIAamp DNA Stool Mini Kit (Qiagen, Haldin, Germany) was used to extract DNA from feces according to the manufacture's protocol (Abd-Alla *et al,* 2007). PCR primers and detection probes were used based on the known episomal repeat sequences for *E. histolytica* (Garfinkel *et al,* 1989); successful amplification and detection of DNA specific to *E. histolytica* and *E. dispar* has been previously verified (Verweij *et al,* 2003a). For the amplification and detection of the internal control, we used PCR primers and a detection probe specific for PhHV-1 (Niesters, 2002). The *E. histolytica*-specific primers and probe set consisted of forward primer histolytica-50F (5'-CATTAAAAATGGTGAGGTTCT T AGGAA-3'), reverse primer *histolyti- ca*-132R (5'-TGGTCGTCGTCTA

GGCAAAATATT-3') and *E. histolytica*-specific double-labeled probe-TEX 615'5TTGACCAATTTACACCGTTG ATTTT TCG GA -3' Iowa Black RQ. The specific primers of *E. dispar* were forward primer *dispar* ('5-GGATCCT CCAAAAAATAAAGTTTTATCA-3') reverse primer *dispar* ('5-ATCCACAG AACGATATTGGATACCTAGTA-3') and *E. dispar* specific double labeled probe (JOE NHS Ester '5-TCTCTGCT ACAACCTC-3' Iowa Black FQ) [Integrated DNA Technology (IDT), Coralville, IA 52241. 1-800-3282661]. PhHV-1- specific primers and probe set consisted of forward primer PhHV-267s (5'-GGGCGAATCACAGATTG AATC-3'), reverse primer PhHV-337 as (5'-GCGGTTCCAAACGTACCAA -3') and specific double-labeled probe PhHV-CY5-'5-TTTTTATGTGTCCG GCCACCATCTGGATC-3'Black Hole Quencher 2 (IDT, USA).

The amplification reactions were performed in a volume of 20ul for each reaction. For 32 reactions (samples) 128*ul* PCR buffer mix (TaqMan master: Roch, Cat. No.04 735 536 001), with 16*ul* 1% BSA, 313.6*ul* H_2O+40 *pmol* (4*ul*) *E. histolytica* primer, 40 *pmol* (4*ul*) PhHV-1primer+80*pmol* (3.2*ul*) *E. histolytica* specific double-labeled probe+32*pmol* (3.2*ul*) PhHV-1-specific double-labeled probe and 5*ul* of DNA sample. Thermo-cycling amplification conditions consisted of 15 min at 95°C followed by 50 cycles of 15 seconds at 95°C, 30 seconds at 60C° and 30 seconds at 72C°, performed by the Roche 2.0 version 4.0 LightCycler PCR Machine (Roche).

ELISA for detection of baboon and human anti-lectin, anti- LC3 and anti-peptide antibodies: ELISA was performed as described previously (Ravdin *et al,* 1990; Abd-Alla *et al,* 2004): purified recombinant 52 kDa LC3 protein (Song et al., 1995), purified native Gal-lectin (Petri *et al,* 1987), or synthetic peptides specific for each of the four putatively protective Gal-lectin heavy subunit IgA epitopes (Abd-Alla *et al,* 2004) were utilized to coat, 96-well microtiter flat-bottomed polystyrene ELISA plates, non-reactive sites were blocked with 1% BSA. The serum was studied at a 1:100 dilution for antibody detection in PBS-Tween (1% BSA); alkaline phosphatase-conjugated goat anti-human IgG (SIGMA, St. Louis, MO) or anti-human IgA antibodies (ICN Biomedicals (Costa Mesa, California) were diluted at 1:5000 to detect IgG and 1:2500 to detect IgA in PBS-Tween 1% BSA at 100 *ul* well and incubated for 2 hours at room temperature. Plate reading and correction for nonspecific background binding were performed as described (Ravdin *et al,* 1990).

For detection of antigen-specific intestinal IgA antibodies, purified native Gal-lectin protein (Petri *et al,* 1987) or a combination of four synthetic peptides were utilized in ELISA (Abo-El-Maged *et al,* 1996; Abd-Alla *et al,* 2004, 2007). Briefly, the flat-bottomed microtiter ELISA plates were coated with lectin protein (0.1 *ug* / well) or synthetic peptides (1ug each peptide per well) and non-reactive sites were blocked with 1% BSA. Fecal samples were mixed with an equal volume of

PBS containing 2 mM PMSF and 100 *ul* / well were incubated for 2 hours at room temperature or overnight at 4°C. Alkaline phosphatase-conjugated goat anti-human IgA antibodies (SIGMA, St. Louis, MO.) were added (1:3000) in PBS-Tween containing 1% BSA for 2 hours incubation at room temperature. Plate reading with correction for non-specific background binding was performed (Ravdin *et al,* 1990).

Statistical analysis: 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). In the current study two sample z tests were used for percentages. 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 and - Z < 1.645 = insignificant changes.

Results

The prevalence of *E. histolytica* and *E. dispar* infection in the baboon population was determined by real time PCR. Of the 54 baboons studied, 37 (68.5%) were infected with *E. histolytica,* 10 (18.5%) with *E. dispar* and only 7(13.0%) were uninfected; mixed infections were not observed. Baboons were more likely to be infected with *E. histolytica* than *E. dispar* ($P<0.01$) or to be uninfected ($P<0.01$). Of the baboons infected with an *E. histolytica,* 84 % exhibited either a serum or intestinal anti-lectin antibody response, compared to only 20% of *E. dispar* infected baboons ($p < 0.001$). None of the uninfected baboons exhibited a serum or intestinal anti-lectin antibody response. In *E. histolytica* infected baboons; 49%, 46%, & 73% respectively had positive ELISAs for intestinal anti-lectin IgA, serum anti-LC3 IgA and serum anti-LC3 IgG antibodies, with the latter being a statistically significant higher percentage ($P<0.01$, Tab. 1).

Table 1: Prevalence of baboon anti-amebic antibodies found after asymptomatic infection in captivity with *E. histolytica* (n=37).

Antibody Tested	ELISA(+)	ELISA(-)
Intestinal Anti-lectin IgA	18 (49%)	19 (51%)
Serum Anti-LC3 IgA	17 (46%)	20 (54%
Serum Anti-LC3 IgG	27 (73%) *	10 (27%)

* $P< 0.01$ compared to prevalence of intestinal anti-lectin IgA or serum anti-LC3 IgA antibodies.

Figs. 1A thorough 1C illustrate relationship between ELISA OD value for each anti-amebic antibody studied and PCR CT value for all 54 baboons studied. There is a strong correlation between having a positive PCR for *E. histolytica* DNA and a positive ELISA OD value for anti-amebic antibodies (Figs. 1A-1C, $P<0.01$). Greater ELISA OD values were observed in antibody-positive *E. histolytica* infected baboons, compared to antibody-positive *E. dispar* infected subjects (Fig. 1A-C, $P<0.01$). Baboons having a negative PCR CT value (>35) all demonstrated a negative ELISA for any of anti-lectin or anti-LC3 antibodies studied (Figures 1A-1C).

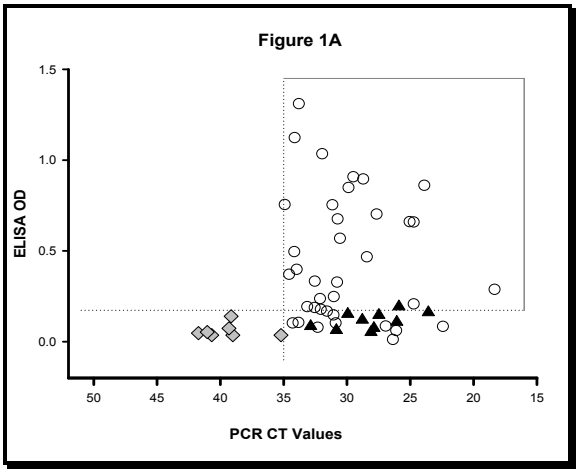
Figure 1A
ELISA OD
1.5
1.0
0.5
0.0
50
45
40
35
30
25
20
15
PCR CT Values

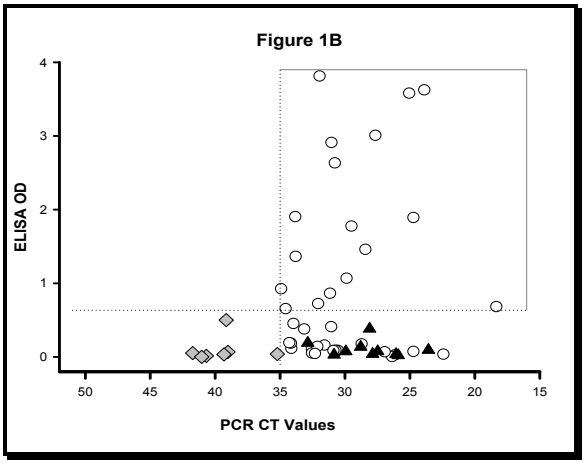
Figure 1B
ELISA OD
4
3
2
1
0
50
45
40
35
30
25
20
15
PCR CT Values

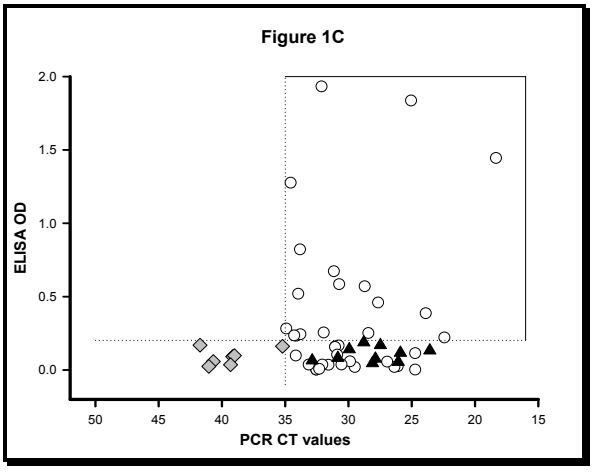
Figure 1C
ELISA OD
2.0
1.5
1.0
0.5
0.0
50
45
40
35
30
25
20
15
PCR CT values

Figure Legend

Fig1: Correlation between ELISA OD values for assay of baboon sera for anti-LC3 IgG antibodies (1A), anti-LC3 IgA antibodies (1B) and for assay of baboon feces for intestinal anti-lectin IgA antibodies (1C) and fecal PCR CT values. A PCR CT value of 35 or less indicates a positive assay for detection of *E. histolytica* or *E. dispar* DNA in feces. All of the baboons with a negative PCR CT value (◊) also had negative ELISAs (OD value below cutoff) for all three of the antibodies studied (1A-1C). There was a positive association with a PCR indicative of a *E. histolytica* infection (○) with the presence of serum anti-LC3 IgG, serum anti-LC3 IgA and intestinal anti-lectin IgA antibodies (P<0.01for each, 1A-1C), but not so for PCRs indicative of *E. dispar* infection (▲), 1A-1C).

For comparison, among 40 human subjects with asymptomatic *E. histolytica* infection, 63% had ELISAs positive for serum anti-LC3 IgA antibodies and 65% for serum anti-LC3 IgG antibodies; comparable results were observed for the 35 human subjects recently cured of amebic liver abscess (Tab. 2).

Table 2: Prevalence of human anti-amebic antibodies found in Durban, South Africa following asymptomatic *E. histolytica* infection or after cure of amebic liver abscess.

Antibody Tested	Asymptomatic Controls (n=40) ELISA+	Amebic Liver Abscess (n=35) ELISA+
Intestinal Anti-lectin IgA	N.A.	N.A.
Serum Anti-LC3 IgA	25 (63%)	20 (57%)
Serum Anti-LC3 IgG	26 (65%)	20 (57%)

Among the baboons that were infected with *E. histolytica* and antibody-positive, it was determined whether they possessed antibodies to a combination of the four Gal-lectin heavy subunit IgA epitope-specific synthetic peptides (Abd-Alla *et al,* 2004, 2006). The baboons with intestinal anti-lectin IgA, serum anti-LC3 IgA and serum anti-LC3 IgG: 83%, 41% & 70% respectively also had positive ELISAs for the peptide epitopes (Tab. 3).

Table 3: Percentage of asymptomatically baboons infected with *E. histolytica*, positive for one of anti-amebic antibodies studied, and recognized the Gal-lectin heavy subunit epitope-specific synthetic peptides on ELISA

Infected Baboons positive for antibody indicated below	(+) Synthetic peptide ELISA
Intestinal Anti-lectin IgA antibodies	15/18 (83%)
Serum Anti-LC3 IgA antibodies	7/17 (41%)
Serum Anti-LC3 IgG antibodies	19/27 (70%)
For any one of the 3 antibodies above	23/31 (74%)

Control studies were performed utilizing the serum anti-LC3 IgA and IgG antibody positive samples from human subjects with asymptomatic infection; 88% and 54% respectively had positive anti-peptide ELISA results. Among the antibody positive human subjects cured of amebic liver abscess, 90% and 80% of subjects respectively exhibited a positive ELISA for anti-peptide IgA or IgG antibodies. In asymptomatically infected baboons and humans who were also either anti-lectin or anti-LC3 antibody positive, 74% and 86% respectively exhibited a positive anti-peptide ELISA (Tabs. 3 & 4) indicat-

ing that baboons share recognition with humans for the same four Gal-lectin heavy subunit epitopes. Although the antibody positive- amebic liver abscess subjects appeared to have a higher percentage of positive anti-peptide ELISA (91%, P=0.275 compared to asymptomatically infected humans), this was not a statistically significant difference (Tab. 4).

Table 4: Percentage of asymptomatic humans with *E. histolytica* infection or cured of amebic liver abscess, positive for one of anti-amebic antibodies studied and also recognized Gal-lectin heavy subunit epitope-specific synthetic peptides on ELISA

Humans positive for antibody indicated below	Asymptomatic Controls, (+) Synthetic peptide ELISA	Amebic liver abscess subjects, (+) Synthetic peptide ELISA
Serum Anti-LC3 IgA	22/25 (88%)	18/20 (90%)
Serum Anti-LC3 IgG	14/26 (54%)	16/20 (80%)
For any of two antibodies above	24/28 (86%)	21/23 (91%)

Discussion

In captivity baboons are ground dwellers (Beaver *et al,* 1988; Munene *et al,* 1998); those included in the current study were housed at OUHSC in large groups, were in full contact with one another and ate fresh fruits and vegetables. The animal caretakers have been the source of outbreaks of amebic infection in non-human primates (Miller *et al,* 1990; Hamlen and Lawrence, 1994); however, we did not investigate that possibility in this study. *E. histolytica* infection has been found to be more frequent than *E. dispar* infection in captivity (Levecke *et al,* 2010), as observed in this study. The findings of a strong correlation between the results for PCR for detection of *E. histolytica* DNA and for ELISA to detect serum and intestinal anti-amebic antibodies further supports the accuracy of PCR using species specific primers and real time PCR (Verweij *et al,* 2003b) for diagnosis and differentiation of *E. histolytica* from *E. dispar* infection in baboons. *Entamoeba* species exhibit variability in pathogenicity among non-human primate hosts; captive Drills (*Mandrillus leucophacaeus*) and Barbary (*Macaca sylvanus*) macaques were found to be asymptomatic carriers of *E. nuttalli*, yet fatal disease resulted when the parasite was transmitted to captive *Colobus guereza* and *Hanuman llangurs* monkeys (Schauerte *et al,* 2010). In addition, to *E. histolytica* (Pang *et al,* 1993; Verweij *et al,* 2003a), other gastrointestinal protozoan are commonly found in non-human primates, including *Giardia* spp. (Peisert *et al,* 1983; Hamlen and Lawrence, 1994; Kalishman *et al,* 1996), *Cryptosporidium* spp. (Go´mez *et al,* 1992; Kalishman *et al,* 1996; da Silva *et al,* 2003) and *Balantidium coli* (Nakauchi, 1999). The clinical presentation of protozoan parasitic infection in the non-human primates varies from loose stools, watery diarrhea and hemorrhagic dysentery to extra-intestinal invasion (liver abscesses), and even death. Outbreaks and deaths caused by *E. histolytica* are frequently reported in non-human primates (Loomis *et al,* 1983; Beaver *et al,* 1988; Marquez-Monter *et al,* 1991; Verweij *et al,* 2003a). Lastly, non-human primates may harbor many

of the same gastrointestinal pathogens as found in humans (Peisert et al., 1983; Hamlen and Lawrence, 1994; Kalishman *et al,* 1996; Go´mez *et al,* 1992; da Silva *et al,* 2003; Nakauchi, 1999) and thus, can be a source for zoonotic infections (Pang *et al,* 1993; Verweij *et al,* 2003a).

Following asymptomatic *E. histolytica* infection, approximately 50% of the infected baboons developed intestinal anti-lectin IgA antibodies and anti-LC3 serum IgA antibodies; over 70% developed serum anti-LC3 IgG antibodies. As this was a point prevalence study, we have no information regarding the duration of *E. histolytica* infection in the baboons. Previously, in human field studies we demonstrated that intestinal anti-lectin IgA antibodies were a more sensitive indicator than a single fecal PCR study of the recent asymptomatic *E. histolytica* infection (Abd-Alla *et al,* 2006). These baboons may have been infected for longer than three to six months, as the prevalence of intestinal anti-lectin IgA antibodies was lower than the serum IgG antibody response, which can be sustained for years (Abd-Alla *et al,* 2006). The point prevalence of serum anti-LC3 IgG and IgA antibodies in a cohort of asymptomatically infected humans and those recently cured of amebic liver abscess were nearly identical to that observed in the baboons. As the immunoglobulin subclasses in baboons, compared to other monkey`s, are most analogous to those of humans (Shearer *et al,* 1999), it is not surprising to observe similar patterns of mucosal and humoral immune responses to *E. histolytica* infections. As expected, *E. dispar* infection did not elicit humoral or mucosal anti-amebic antibody responses in the baboons; a finding concordant with human clinical studies (Jackson *et al,* 1985; Abou El-Maged *et al,* 1996; Ravdin *et al*, 1990, 2003; Abd-Alla *et al,* 2000; Haque *et al,* 2001).

When evaluating the recognition of the four heavy subunit epitope-specific synthetic peptides by baboon and human anti-amebic antibodies, the percent positive ELISAs for anti-peptide antibodies were comparable in asymptomatically infected baboons and humans, indicating that *E, histolytica* infection of baboons elicited antibodies with shared Gal-lectin heavy subunit epitope specificity. These findings correlate with studies of experimental intranasal immunization of baboons with these same epitope-specific synthetic peptides, which elicited intestinal IgA and humoral IgA and IgG antibodies that recognized the native Gal-lectin, bound to the surface of viable *E. histolytica* trophozoites and inhibited the in vitro galactose-sepecific adherence of trophozoites to Chinese hamster ovary cells (Abd-Alla *et al*, 2007).

Of interest, the seropositive subjects recently cured of amebic liver abscess had a higher percent of positive ELISA for the peptide epitopes and a higher OD value for anti-LC3 antibodies**.** Previously, the present authors demonstrated that over 36 months of follow up, ELISA OD values for anti-amebic antibodies are higher in liver abscess cohort, compared to antibody positive controls (Abd-Alla *et al,* 2006).

Conclusion

Asymptomatic *E. histolytica* and *E. dispar* infections occur in baboons while in captivity. Asymptomatic *E. histolytica* infection of baboons elicits anti-amebic anti-bodies with prevalence analogous to that observed during asymptomatic infection in humans. Baboon and human antibodies recognize native Gal-lectin, a Gal-lectin heavy subunit-based recombinant protein (LC3) and Gal-lectin heavy subunit based epitope-specific synthetic peptides. Given that other experimental animal models of *E. histolytica* infection utilize hosts that aren`t subject to natural infection by the parasite and have immune systems that may not produce the analogous epitope-specific antibody responses, baboons may be a more appropriate experimental animal host for the study of immune response to *E. histolytica* infection and to determine the efficacy of the experimental amebiasis subunit vaccines. The baboons naturally infected with *E. histolytica* while in captivity develop serum and intestinal anti-Gal lectin antibody responses that were found to recognize the same four Gal-lectin heavy subunit epitopes as antibodies from humans who were asymptomatically infected or recently cured of ALA. Thus, the baboons constitute an ideal experimental host for study of *E. histolytica* infection, mucosal immunoresponses and the efficacy of an amebiasis subunit vaccine.

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