# Immunogenicity Of Entamoeba histolytica Crude and Fractionated Antigens in Animal Model

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## Abstract

**Received 19/Nov/2002, Accepted 7/Nov/2003**

***Introduction:*** The immunogenicity of crude amoebic antigen and its fractions prepared from Entamoeba histolytica (NIH:200) was evaluated in experimental animals.

***Material and Methods:*** Forty two guinea pigs of either sex free from Entamoeba infection and aged around 3 to 4 weeks were randomly divided into 5 groups. The treated groups consisted of 8, 10, 6, and 8 animals and 10 animals served as controls. Crude amoebic extract and its chromatographed fractions were used to immunize the treated animals. All the animals were assessed for immunity status, challenged with Entamoeba trophozoites and subsequently examined for lesions of the caecum and liver.

***Results:*** Of the 8 animals immunized with crude antigen, one had liver abscess and 5 had caecal lesions. None of the 10 animals immunized with fraction I (FI) had hepatic lesions and one had caecal lesions. Both caecal and hepatic lesions were observed in animals immunized with FII & FIII.

***Conclusion:*** Results show that vaccination with the F1 fraction of Entamoeba histolytica provided up to 90% protection against infection. The other fractions and the crude extract provided less protection.

**Key words:** Entamoeba histolytica, Crude Amoebic antigen, Fractionated Amoebic antigens, protection, Guinea pigs

## Introduction

Amoebiasis due to Entamoeba histolytica is world-wide in its distribution and has a spectrum of clinical manifestations (1). Entamoeba histolytica is a protozoan parasite that causes amoebic colitis and liver abscess in developing countries such as Mexico and India (2). Most often, infection is symptomless but in approximately 10% of human hosts, invasion of gut mucosa and extra intestinal sites leads to dysentery, amoebic liver abscess, pulmonary abscess and involvement of other organs (3). In spite of effective treatment the reported morbidity and mortality due to amoebic infection justify efforts to limit or eradicate the disease by vaccination (4). The immuno-pathology of hepatic amoebiosis has been studied by inoculation of trophozoites in experimental animals (5). In humans there is some degree of protection after recovery from acute amoebic colitis (6) and it has been observed that recurrence of amoebic liver abscess following eradication of the infection is rather unusual (7). However there is not yet sufficient evidence to justify large scale human trials of immunization procedures (8, 9). The present study was carried out to see if prior immunization of guinea pigs would sufficiently sensitize the immune apparatus of the host to resist a fulminant amoebic infection.



## Material and Methods

### * *Experimental animals*

Forty two guinea pigs of either sex, aged around 3 to 4 weeks and free of infection by amoeba were used for this study. Animals were divided into 5 groups. Ten animals of the first group did not receive any antigen. Eight animals of the second group were treated with crude amoebic extract; ten animals of the third group were treated with fraction I (FI); 6 animals of the fourth group were treated with FII and 8 animals of the fifth group were treated with FIII.

### * *Antigens*

Entamoeba histolytica (NIH:200) was grown axenically in TPS-I medium in the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Crude extract was prepared after ultrasonication of the trophozoites. Three chromatographed fractions (column chromatography using sephadex G-200 beads) of the crude extract were collected and labelled as fraction I (FI), fraction II (FII) and fraction III (FIII).

### * *Inoculation schedule*

0.3 ml of the different amoebic antigens, containing 0.84 mg amoebic protein mixed with equal volume of Freund's Complete Adjuvant (FCA), was used for inoculation of the guinea pigs subcutaneously, thrice, at one week intervals.

### * *Challenge*

The inoculated and control group animals were challenged intracaecally with $8 \times 10^4$ to $8 \times 10^5$ Entamoeba trophozoites in 0.5 ml. Suspension. The guinea pigs were sacrificed 7 to 10 days later for histopathology examination. Normal liver histology and absence of amoebic trophozoites were regarded as criteria of protection.

### * *Immune status*

The immune status of the animals was evaluated by a dermal sensitivity test and the level of anti-amoeba antibody in the serum of the animals.

In the dermal sensitivity test 0.1 ml of amoebic antigen containing 40$\mu$g amoebic protein was injected intradermally. Normal saline was used for control. The reaction to antigen was compared with control after 30 minutes and at intervals of 2, 24 & 48 hours. Erythema with induration of more than one cm. diameter was interpreted as a positive reaction. The skin test remained positive in many animals for six days after intradermal injection of the antigens.

An ELISA technique was used to detect the presence of antibody in the sera of the experimental animals using different amoebic antigens one week after challenge.

## Results

The immune status of the five groups of animals, in terms of skin reactivity and antibody titre, and their response to challenge with E histolytica sporozoites

are shown in Table I.

In Group 1 (control) all 10 animals had no antibody titre and were negative to the intradermal test. After challenge with sporozoites 9 nine of the 10 animals had caecal lesions and 5 had hepatic lesions.

In Group 2 (crude antigen) 5 of 8 animals had an antibody response and 5 had a positive intradermal test. After challenge 5 had caecal lesions and one of these also had hepatic lesions. Three of the animals with lesions had an antibody titre and two had a positive intradermal reaction.

In Group 3 (FI) 7 of 10 animals had an antibody titre and a positive intradermal reaction. Only one animal had a lesion and this animal was negative for antibody titre and intradermal test.

In Group 4 (FII) three of the 6 animals had an antibody titre and three a positive intradermal reaction. After challenge 5 animals had lesions including 3 that had an antibody titre and a positive intradermal reaction.

Table 1: The effect of immunization with crude antigen, FI fraction, FII fraction and FIII fraction of Entamoeba hystolytica on the immune status and subsquent response to challenge with trophozoites.

| Group | no. | Immune State | | | Response tochallege | |
|---|---|---|---|---|---|---|
| | | No-positive for antibody | No.positive to intradermal | Total no. positive | No.with Caecal lesions | No.with hepatic lesions |
| Control | 10 | 0 | 0 | 0 | 9(90%) | 5(50%) |
| Crude Antigen | 8 | 5 | 5 | 6 | 5(62.5%) | 1(12.5%) |
| FI | 10 | 7 | 7 | 8 | 1(10%) | 0 |
| FII | 6 | 3 | 3 | 4 | 5(83.3%) | 1(16.7%) |
| FIII | 8 | 5 | 5 | 5 | 5(62.5%) | 1(12.5%) |

In Group 5 (FIII) 5 of 8 animals had an antibody titre and 5 had a positive intradermal reaction. Five animals had lesions and two of these had an antibody titre and positive intradermal reaction.

## Discussion

The induction of immunity by live trophozoites has been observed by Jain et al (10) who reported that immunization of guinea pigs with a low grade infection either intracaecally or intramesentrically conferred protection on 70 percent of animals against an intracaecal challenge with virulent isolates of Entamoeba histolytica.

There was a good correlation in the immunised animals between the antibody titre in the serum and the intradermal test indicating effective stimulatiom of the immune system.

If an antibody titre and a positive intradermal test are considered as a measure of immune response to inoculation, the proportion of responders was 75% to crude antigen, 80% to FI, 66.7% to FII, and 62,5% to FIII. If the proportion of animals without lesions after challenge is used as a measure of protection, the protection afforded by the inoculations was 37.5%, 90%, 16.7% and 37.5% respectively for crude antigen, FI, FII and FIII respectively. Although antibody was detectable in the majority of animals it is apparent that the presence of antibody is not highly indicative of protection. These results agree with those of Sawhney (11) who reported 90% protection with FI and 33% protection with FIII. Krupp (12) using an inoculation dose of 5.6 mg protein per animal compared to 2.5mg used in these experiments, obtained 100% and 70% protection from FI and FIII respectively. The amount of protein in the inoculation dose seems to be critical. The finding reported here that the presence of antibody is not an indication of protection accords with the conclusion of Krupp (6).

The results reported here show that when used in experimental animals fraction FI antigen of Entamoeba histolytica stimulates good antibody production and provides better protection than crude antigen or fractions FII or FIII. The crude antigen is preferred for diagnostic purposes because the preparation of FI is a cumbersome and time consuming procedure.



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