INTRODUCTION

Amoebiasis is the infection of humans due to the intestinal protozoan *Entamoeba histolytica*. The motile form of the parasite i.e. the trophozoite, usually lives as a commensal in the lumen of the large intestine, where it multiplies and differentiate into the resistant form, the cyst, responsible for the transmission of the infection. As a commensal, *Entamoeba histolytica* has a world wide distribution and produces no signs or symptoms. The causation of invasive amoebiasis by pathogenic *E. histolytica* include

1). colonization of the gut;
2). intimate contact or adherence to the intestinal mucosa;
3). disruption of intestinal barriers by enzymes or toxic products; and
4). lysis of intestinal cells and host inflammatory cells, leading to colonic ulcers, deep tissue or liver invasion (Ravdin, 1986).

Taxonomic Classification

*Entamoeba histolytica* Schaudinn, 1903 belongs to the family Entamoebidae of the order Amoebida, Subclass Gymnamoebia, class Lobosea, superclass Rhizopoda of the subphylum Sarcodina under the phylum Sarcomastigophora (Levine et al 1980).

Life cycle

The complete life cycle of *E. histolytica* consists of four consecutive stages; namely, trophozoites, precyst, cyst and metacyst. Trophozoites multiply by binary fission and encyst
during the commensal stage; after two successive nuclear divisions, they produce typical quadrinucleated cysts protected by a chitin containing rigid wall. A single quadrinucleated metacystic amoeba escapes from each cyst to produce, after division, eight uninucleate amoebae (Dobell, 1926; Cleveland and Sanders, 1930; Tanabe, 1934).

Prevalence

The estimation of the magnitude of the global problem of annual morbidity and mortality due to amoebiasis is not clearly known. While the best available data remain imprecise and limited because of currently inadequate diagnostic tools, it can reasonably be estimated that there are nearly 500 million amoebic infections worldwide, with 8-10% of these infections causing predominant clinical disease in Asia, Africa and Latin America (Walsh, 1986). The estimated mortality, morbidity and prevalence reflects amoebiasis as the third leading parasitic cause of death on a global scale, behind only malaria and schistosomiasis (WHO, 1969; Walsh, 1986).

In India, studies have been made in different areas including both rural and urban, in slum areas, selected communities and hospital patients. Stool positivity ranges from 0.5 to 38% in urban areas and around 8 to 30% in rural areas (Rao et al 1971; Sanyal et al 1972; Mathur and Kaur, 1974; Arora et al 1976; Kulkarni et al 1978; Tamboli and Sharma, 1979; Veerannan, 1979; Joshi et al 1980; Nanda et al 1984). In West Bengal, a study regarding incidence of *E. histolytica* in hospital patients was carried out for seven years in Bankura district by Sengupta and Bhattacharya (1975). These investigators has shown that about 27.3% people were found to carry amoebic infection in a sample survey based on 18,788 cases.
In Calcutta, the percentage of faecal samples containing *E. histolytica* trophozoites and/or cysts was 10% whereas the percentage of individuals with past or present invasive amoebiasis, as measured by the indirect haemagglutination test (IHA) and the fluorescent antibody test (FAT) was 81% (Meerovitch et al 1978).

**Biologic characteristics**

The trophozoites of *E. histolytica* are microaerophillic, uninucleate organisms having a double layered (120 Å) limiting membrane. A 20-30 nm glyocalyx is present (Lushbaugh and Miller, 1974) surrounding the uroid area with vesicles external to the cell membrane (Fletcher et al 1962; El-Hashimi and Pittman, 1970; Pittman et al 1973). The trophozoites require a low pH (6.0-6.5) and complex medium for growth. *E. histolytica* has microfilament-like structures and actin, but no evident cytoplasmic microtubules and mitochondria (Rosenbaum and Wittner, 1970; Von Brand, 1973; Harlow et al 1976; Aust-Kettis et al 1977). Typical electron transport mechanisms are not present as evident by lack of cytochromes and functional tricarboxylic acid cycle (Hilker and White, 1959; Weinbach and Diamond, 1974).

The surface of trophozoites have Concanavalin A receptors and negative to neutral overall surface charge (Martinez-Palomó et al 1973; Bos and Vande Griep, 1977; Trissl et al 1977). Presence of many phagocytic and acid phosphatase staining lysosomal vacuoles have been reported (Weinbach et al 1976). Presence of an unusual phospholipid-ceramide amnioethyl phosphonate which is a phospholipase resistant species was found in the plasma membrane enriched with cholesterol (Aley et al 1980). The trophozoites also contain highly active thiol-containing proteases active over a broad pH range (Jarumilinta and Kradolfer, 1964; McLaughlin and Faubert, 1977).
Pathophysiology

Early studies of amoebiasis were mainly done with autopsy material, and led to the general opinion that inflammation is mild or absent in amoebic lesions that are free of pathogenic bacteria where the degree of inflammation does not correlate with the vigorous necrotic processes (Castro, 1974; Kagan, 1974). Inhibition of monocyte chemotaxis by *E. histolytica* (Kretschmer et al 1980) and reduced phagocytic activity of peripheral polymorphonuclear leucocytes from patients with amoebiasis (Ghosh and Sen, 1980) have been observed. On the other hand, inflammatory processes do occur (Brandt and Perez Tamayo, 1970) as indicated by elevated titres of complement (Flores-Barroeta et al 1970) and leucocytosis (Aikat et al 1978). It is known from biopsy studies that acute intestinal amoebiasis is accompanied by generalized haemorrhagic inflammation of the mucosa, with pronounced edema and infiltrations by neutrophils and eosinophils (Rao et al 1975; Tandon et al 1975). Furthermore, in chronic amoebiasis and in amoeboma, chronic and allergic types of inflammation with infiltrates of lymphocytes, histiocytes, and eosinophils have been described (Parodi, 1958; Anh, 1971; Sharma et al 1972).

Antigens of *E. histolytica*

The elucidation of antiamoebic immune reactions is complicated by the large number of antigenic components that may evoke an equally large number of different immune responses. Studies regarding the trophozoite antigen, those exposed on the surface have created special interest since they may induce effector mechanisms lethal to the parasite. Such antigens have been demonstrated by the surface binding of fluorescence labelled immune sera (Biagi et al 1966), by the abrogation of surface binding after absorption of immune sera with
trophozoites (Boonpucknavig et al 1967) and by antibody mediated lysis of trophozoites by complement (Ortiz-Ortiz et al 1978; Huldt et al 1979). Surface antigens have been shown to contain carbohydrates as they bind to Concanavalin A (Parkhouse et al 1978) however, it has not yet been demonstrated whether these antigens are identical to the glycoproteins isolated from amoebic plasma membranes (Aley et al 1980). On the other hand, carbohydrate-free membrane antigens of \textit{E. histolytica} have also been described (Serrano et al 1977).

A surface antigen of \textit{E. histolytica} have been identified and partially characterized by Torian et al (1986) who used monoclonal antibodies to precipitate an intrinsically radiolabelled 96,000 Dalton antigen which reacted with the patient's sera. More recently the 96,000 Dalton antigen was also reported to be detectable in \textit{E. histolytica} trophozoites belonging to the both pathogenic and non pathogenic zymoderes (Torian et al 1989).

The rapid turn over between the surface and internal membranes in \textit{E. histolytica} trophozoites confers some doubt about the origin of the surface antigens. Antigenic determinants may be located in the recycling cytoplasmic and plasma membranes (Aust-Kettis and Sundqvist, 1978). The effective immunogenicity has also been observed in lysosomal and ribosomal fractions of the trophozoites (Boonpucknavig et al 1967; McLaughlin and Meerovitch, 1975). An extensive comparison of amoebic antigens (homogenates and extracts of whole amoebae) and human immune sera from different parts of the world has revealed at least 14 precipitating antigens and the similarity of bands suggest the existence of a common antigenic backbone in different strains of \textit{E. histolytica} (Krupp, 1966; Chang et al 1979).

Antigens released from live amoebae may play a role in the pathology of amoebiasis by toxic reactions or by the formation
of immune complexes (Trissl, 1982). Other potentially antigenic constituents includes a glycosidase (Lundblad et al 1981) and a micro exudate that trails behind the amoebae on the culture substrate in vitro (Pinto Da Silva et al 1975). However, it would appear that the surface antigen described by Torian et al (1986, 1989) is perhaps one of the major antigenic fractions responsible for the immunological response in human infection (Jalan and Maitra, 1988).

**Cellular response in amoebiasis**

Accumulated experimental evidence strongly indicates that cellular immunity plays an important part in controlling the exacerbation of invasive amoebiasis. It has been found that intradermal vaccination of inbred hamsters with live axenic *E. histolytica* (resulting in a superficial ulcer which later healed) protected the animals from a subsequent intrahepatic challenge (Ghadirian and Meerovitch, 1978). The same results were obtained when a high molecular weight fraction of the amoebic extract was used as the vaccine (Ghadirian et al 1980). Thymectomy or anti T-Cell treatment of hamsters resulted in the production of bigger liver abscesses and an increase in the metastatic spread of the amoebae (Ghadirian and Meerovitch, 1981). The same results were obtained in animals treated with silica or antimacrophage serum but not with BCG (Ghadirian and Meerovitch, 1982; Ghadirian et al 1983) which confirmed the assumption that the protection was due to the activation of cellular immunity, possibly through the mediation of the T cell-macrophage system (Meerovitch and Smith, 1984). In vitro, lymphocytes, peritoneal and spleen cells from vaccinated or protected hamsters were shown to be able to kill trophozoites of *E. histolytica*, while such cells from hamsters with amoebic liver abscess were not (Ghadirian and Meerovitch, 1982).
The experimental evidences have shown that cell-mediated protective immunity against invasive amoebiasis can be raised by means of sensitization with amoebae or amoebic extract, and that interference with the T cell-macrophage system can abrogate it (Meerovitch and Smith, 1984).

It has also been observed in human situation, that patients cured of amoebic liver abscess have an E. histolytica antigen-specific T lymphocyte proliferative response in vitro, producing lymphokines (including gamma interferon) that elicited in vitro amoebicidal activity in human monocyte-derived macrophages (Salata et al 1986, 1987).

**Humoral response in amoebiasis**

In amoebiasis, antibodies are produced in response to exo-antigens produced by the amoebae (metabolites, toxins, lectins, enzymes) or endo-antigens liberated upon the death and disintegration of the amoebae (structural proteins and endogenous enzymes). An antibody response in the serum is regularly observed in symptomatic as well as asymptomatic amoebiasis and the titre of antibody in an endemic area may be similar in both groups. There is a general correlation between the stage of infection and the presence of antibodies both by titres and immuno electrophoretic patterns. Acute invasive amoebiasis generally provokes high serum titres and complex patterns, whereas asymptomatic or previously symptomatic amoebiasis leads to lower titres (Neal et al 1968; Krupp, 1970; Krupp and Powell, 1971). It has also been reported that the antibody response may persist from 2-11 years and the clinical outcome is not related to the titre of antibody (Kessel et al 1965; Kotcher et al., 1970; Juniper et al., 1972; Patterson et al., 1980).
It is generally assumed that serum antibodies to *E. histolytica* appear only after invasion by the parasite (Elsdon-Dew, 1970, 1975). The relatively high frequency of positive sera in asymptomatic patients who pass cysts is thought to result from subclinical invasion (Savant et al 1974). Sensitive indicators of a local humoral response are locally secreted coproantibodies and the release of such coproantibodies have been demonstrated in intestinal amoebiasis (Shaalan and Baker, 1970; Mahajan et al 1972; Sharma et al 1978). In contrast to serum antibodies, coproantibodies persist for only a short time (Martinez Cairo et al 1979).

In areas endemic for *E. histolytica* various studies on the serum levels of the major immunoglobulin classes have been performed repeatedly (Perches et al 1970; Abioye et al 1972; Ravi et al 1975; Ganguly et al 1978). The levels of IgG seems to be elevated in amoebiasis although increased levels of IgM and IgA though smaller than IgG, have also been reported (Ravi et al 1975). The intimate involvement of IgG was indicated by the rapid fall of its concentration during chemotherapy (Abioye et al 1972). Since it was unclear whether the rise of IgG levels in amoebiasis was due only to specific antibodies to *E. histolytica*, at least part of the increase could be attributed to the specific response (Yap et al 1970; Sepulveda et al 1972). Revoltella et al (1980) studied anti-amoebic IgE in the sera of patients with a mixed infection consisting of helminthes, *Escherichia coli* and *E. histolytica*. However anti amoebic IgE was not found in any sera in the absence of anti-helminthic IgE, suggesting the non-specific induction of anti amoebic IgE by the helminths.

The realization that antibodies are produced in invasive amoebiasis, coinciding with the advent of better antigens, and advances in serological methods, has led to the wide spread
interest in the use of immunodiagnostic techniques in amoebiasis. Serum antibodies can be demonstrated by commonly used serologic techniques (Kessel et al 1965; Maddison et al 1968; Morris et al 1970; Bos and van den Eijk, 1976; Thomas et al 1981) and by the amoeba-specific immobilization test (Biagi and Buentello, 1961). These methods appear to be highly specific for amoebic antibodies, but vary in sensitivity. Since antibodies may persist for an ill-defined period after termination of infection, a positive serological result is not in itself an adequate basis for the diagnosis of active amoebic infection. However, failure to detect antibodies by the more sensitive methods help the physician to rule out appreciable tissue invasion.

**Immune Complexes**

The prolonged persistence of antibodies even after recovery may depend on the undetected persistence of antigen or infection and clearly, the availability of an inexpensive, rapid and sensitive serologic assay to identify patients with invasive disease would be of great value. One approach to such a test was to identify serologically reactive antigens either in free or complexed (i.e. with antibody) form leading to the formulation of a defined antigen assay system.

Antigen-antibody complexes are regularly found in patients with certain disorders and there is compelling evidence for the view that such complexes may play a pathogenic role (W.H.O. 1977). Circulating immune complexes are likely to be detectable in acute phase of any infectious disease and thus their presence in a disease may not always have pathogenic importance. However, demonstration of persistent immune complexes may be important as it may indicate an underlying immunological dysfunction at the lymphocyte or phagocyte level, which hinders the clearance of
these complexes from the circulation. Immune complexes are formed by the union of one or more antibody molecules with one or more antigen molecules. Once formed, the complex may activate cullular and humoral mediator systems that cause tissue injury. Many human diseases can now be attributed to the presence of immune complexes in tissues. Some examples are systemic lupus erythematosus, acute and chronic glomerulonephritis, rheumatoid arthritis, vasculitis, and tissue injury associated with a variety of infections agents (Wiggins and Cochrane, 1981).

Several methods have been devised for the detection of complexes in sera and other body fluids, some of which are based on physicochemical characteristics such as increased sedimentation coefficient of the component molecules (Kunkel et al 1961), decreased solubility in the cold (Cream, 1977) or polyethylene glycol solutions (Gangeot et al 1978) while others use binding to natural receptor molecules such as Clq (Hay et al 1976), C3 receptors on Raji cells (Theofilopoulos et al 1976) or Fc receptors on macrophages (Onyewotu et al 1975). However, correlations among these tests using the same principle (Lambert et al 1978) were poor which may be due to the fact that different tests detected different levels of immune complexes. (Chia et al 1979).

Among the parasitic infections, circulating immune complexes (CIC) have been documented in malaria (Houba et al 1970), trypanosomiasis (Franklin et al 1957), Schistosomiasis (Natali and Cioli, 1976), onchocerciasis (Ngu and Blackett, 1977) and toxoplasmosis (Shahin et al 1974). Increased soluble immune complexes have also been documented in amoebic liver abscess patients and chronic cyst passers (Onyemelukwe and Onyewotu, 1981; Nuti et al 1982; Pillai and Mohimen, 1982; Vinayak et al 1986). Pillai and Mohimen (1982) reported the existence of immune complexes in the sera of large number of patients with colonic amoebiasis and particularly those with
systemic manifestations. These immune complexes were further shown to contain a detergent extractable *E. histolytica* membrane protein having a molecular weight of 35,000 Daltons. Moreover, an antigen assay has also been devised using affinity-depleted and affinity-purified antibodies which could serve as a specific and sensitive marker of invasive amoebiasis which further showed the preferential deposition of such specific immune complexes in the colon and liver (Jalan and Maitra, 1988).

Since the demonstration of the presence of amoebic antigen in CIC, may discriminate the present from past infection, a kinetic study regarding the formation, deposition and clearance of such CIC in experimental animal models is inevitable. Therefore, the present investigation was directed to approach such objectives using a widely used model of hepatic amoebiasis in golden hamsters.

**Aim of the present study**

1). To establish a model of hepatic amoebiasis in golden hamster using a local isolate of *Entamoeba histolytica*.
2). To determine the level of anti *E. histolytica* antibody in the sera of infected animals at graded interval.
3). To demonstrate specific CIC in the sera of infected hamsters by different methods and to evaluate the diagnostic applicability of the findings.
4). To study the formation, circulation and preferential deposition (if any) of immune complexes in the tissues of infected hamsters in relation to the progressive disease process.
Importance of the present study

The inadequateness of the present serologic tests to differentiate between past and present amoebic infection by demonstration of anti-amoebic antibodies particularly in an endemic area needs a marker of disease activity. A kinetic study of *E. histolytica* associated immune complexes in an animal model and use of a specific and sensitive immunoassay for *E. histolytica* antigens would be of particular value in the following situations.

1). to establish and evaluate clinical diagnosis of amoebiasis.
2). to study the sequelae of *E. histolytica* infections in humans.
3). to study the secondary inflammatory role of immune complexes in amoebic infections.
4). to screen for the expression of *E. histolytica* proteins in recombinant bacterial clones using the system as a tool in molecular biology.