CHAPTER
ONE

INTRODUCTION
1. INTRODUCTION

Amoebiasis, caused by an enteric protozoan *Entamoeba histolytica*, is one of the major parasitic diseases of mankind. Human intestinal amoebae were first reported from India by Lewis in 1870. Losch (1875) was the first to recognize the pathogenic role of amoebae in the stool of a patient suffering from chronic dysentery. Epidemiological studies revealed that the parasite infects about 10% of the world population, which works out to about 50 million cases of invasive colitis or liver abscess leading to 50,000 to 100,000 deaths every year (Walsh, 1986). The prevalence rate is higher in developing countries than in developed countries. In India, the prevalence of *E. histolytica* infection ranges from 3.6% to 47.4% in different parts. A similar observation has been noticed in communities of high and low socio-economic status (WHO Scientific Working Group, 1984).

The disease caused by the parasite manifests diverse clinical symptoms ranging from asymptomatic carrier state to acute amoebic dysentery and amoebic liver abscess. Martinez-Palomo and Gonzales-Robles (1973) were the first to correlate symptomatic and asymptomatic amoebiasis cases with virulent and avirulent forms of *E. histolytica* by a lectin, i.e., concanavalin A agglutination pattern. Reeves and Bischoff (1968) and later Sargeaunt and Williams (1978b) documented similar differences on the basis of zymodeme patterns. They suggested that the isoenzyme profile of any given strain was an inherited marker of pathogenicity which could be used to reliably predict the clinical outcome of infection. However, the hypothesis was not supported by Mirelman *et al.* (1986) who suggested that the characters of pathogenic and nonpathogenic *E. histolytica* are variable and change in presence or absence of bacteria. With the advent of new biotechnological methods such as DNA probes and monoclonal antibodies, it is now possible to differentiate pathogenic and nonpathogenic strains of *E. histolytica* (Strachan *et al.*, 1988; Garfinkel *et al.*, 1989; Tannich *et al.*, 1989; Bracha *et al.*, 1990; Merino *et al.*, 1990; Tachibana *et al.*, 1990; Gonzalez-Ruiz *et al.*, 1992; Reed *et al.*, 1992). These approaches will further help in revealing the
pathogenic role played by the parasite in different disease conditions ranging from asymptomatic to acute disease.

The diagnosis of amoebiasis is mainly carried out on the basis of clinical symptoms, detection of the parasite, serological findings and histopathological observations. Demonstration of trophozoites or cysts in the stool samples is the only definitive test. However, the limitation of this technique is that it is time consuming, requires expertise and, therefore, cannot be used for large scale screening. Moreover, patients having extra-intestinal complications do not show the presence of cysts/trophozoites in stools. Under these circumstances, indirect methods such as serological assays are found to be more useful. A battery of tests have been used by various workers. Each test has its own limitation in terms of specificity and sensitivity. It was also suggested that the reproducibility and specificity of the tests mainly depend on the quality of antigen used. In 1985, the WHO Expert Committee on Amoebiasis strongly recommended the application of a standard antigen for obtaining reproducible results in the serodiagnosis of amoebiasis. (WHO Scientific Working Group, 1985).

Systemic humoral immune response occurs in invasive amoebiasis and are heralded by the prompt increased level of specific circulating antibodies, predominantly the IgG class and to a lesser extent, the IgM, IgA and IgE classes (Trissl, 1982). Virtually all invasive pathogenic trophozoites are resistant to complement-mediated lysis. However, the role of complement in preventing initial invasion of the colonic mucosa is uncertain. The increased level of antibodies as a result of humoral immune response followed by invasion of *E. histolytica* neither cures the invasive process nor does it seems to prevent subsequent reinfection (Ackers, 1982). Cell mediated immunity appears to play an important role in the acquired immunity of amoebiasis, especially in liver abscess (Salata *et al.*, 1985a & b, 1986a & b).

Multiple antigenic determinants of *E. histolytica* present on the surface as well as in the cytosolic components have still not been fully characterized (Parkhouse *et al.*, 1978; Aley...
et al., 1980). Preliminary work on antigenic analyses using sera from immune or infected individuals have indicated that the whole amoebic extract prepared from *E. histolytica* trophozoites are heterogenous in nature (Trissl, 1982). With the advent of hybridoma technique (Kohler and Milstein, 1975) it is now possible to purify specific antigen molecules to homogeneity which has enabled detailed characterization of the purified molecules by biochemical and immunological parameters. Use of monospecific antibodies are now found to be an ideal tool in the development of an assay system which could easily and specifically detect the antigen present in the diseased person.

Use of monoclonal antibodies for purification and characterization of various surface as well as cytosolic antigens of *E. histolytica* have been reported recently (Ortiz-Ortiz et al., 1986; Torian et al., 1990a & b; Petri et al., 1990a & b; Tannich et al., 1991a; Reed et al., 1992). Monoclonal antibody based *E. histolytica* antigen detection systems have also been reported (Merino et al., 1990; Wonsit et al., 1992). Recently monoclonal antibodies to surface associated adhesive molecules of pathogenic strains of *E. histolytica* and their role in differentiation between pathogenic and nonpathogenic isolates of *E. histolytica* have been documented (Bhattacharya et al., 1990; Tachibana et al., 1990; Vinayak et al., 1990).

Protective immunity against intrahepatic *E. histolytica* inoculation was induced in adult hamsters using live trophozoites or crude monoxenic or axenic *E. histolytica* antigen plus adjuvant (Sepulveda et al., 1973; Ghadirian and Meervitch, 1978). Major protection against the high molecular weight (660 kDa MW, glycoprotein) fraction antigen were demonstrated in guinea pigs and hamsters (Krupp, 1974b; Ghadirian et al., 1980; Vinayak et al., 1980. Das et al., 1993).

Recently, a 260 kDa galactose specific adherence protein of *E. histolytica* which contains highly conserved B- and T-cell epitopes, which showed a high degree of vaccine efficacy in the gerbil model of amoebic liver abscess, has been documented (Ravdin et al., 1993). However, till date no effort has been made to produce and characterize monoclonal antibodies against axenic *E. histolytica* antigens which could be exploited for specific and
reliable diagnosis and for immunoprophylaxis against the disease.

In view of the above, the present study was designed with the following major objectives:

* To prepare and analyse, biochemically and immunologically, the complex nature of soluble antigens of axenic *Entamoeba histolytica* (NIH:200).
* To purify and determine the most immunogenic and protective fraction of crude soluble antigen by different biochemical and immunological procedures and to conduct *in vivo* challenge experiments using the above identified fractions.
* To develop and characterize specific monoclonal antibodies (MAbs) raised against highly immunogenic and protective fraction of soluble *E. histolytica* antigens with the intention of developing a test system which could quickly and specifically detect the present cases of amoebiasis.
* To directly demonstrate the MAb specific polypeptide in amoebic cases.
* To Purify the MAb specific antigenic polypeptide by immuno-affinity column chromatography and to demonstrate homogeneity of the polypeptide by SDS-PAGE.
* To analyse biochemically and immunologically the nature of purified antigen and its relevance to the immune response and in immunoprophylaxis.