ANTIGENS OF DIAGNOSTIC AND PROPHYLACTIC IMPORTANCE IN *ENTAMOEBA HISTOLYTICA*

S. Bhattacharya, K. Sengupta, S. Sengupta, P. Mukhopadhyay, A. Debnath and P. DAS*

Division of Microbiology, National Institute of Cholera and Enteric Diseases, P-33, CIT Road, Scheme-XM, Beliaghata, Calcutta-700 010.

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Amoebiasis, caused by *Entamoeba histolytica*, is one of the major parasitic diseases of mankind. Epidemiological statistics suggest that the parasite infects about 10% of the world population, which works out to 50 million cases of invasive colitis or liver abscess leading to 50,000 to 100,000 deaths every year(1). In India, *E. histolytica* has been detected from 2-38% of population(2). The disease caused by the parasite, manifests diverse clinical symptoms ranging from asymptomatic carrier state to acute amoebic dysentery and amoebic liver abscess which clearly suggest the presence of two distinct forms of parasite, one that causes the disease and other that thrives as an asymptomatic carrier due to its nonpathogenic nature. The diagnosis of amoebiasis is done by microscopic examination, serological findings and histopathological observations. Till now, demonstration of trophozoites or cysts in the stool samples is the only definitive test. However, the limitation of this technique is, time consuming, requires skilled personnel and depends on the number of cysts and trophozoites excreted at the time of examination which, therefore, cannot be used for large scale screening. Similarly, a battery of serological tests have been devised by various workers but, each test was found having its own limitation in terms of specificity and sensitivity. It was also noticed that the reproducibility and specificity of the tests mainly depend on the quality of antigen used. Apart from the diagnostic dilemma,

questions like, diagnosis and differentiation of pathogenic *E. histolytica* from non pathogenic *E. dispar* directly from the clinical specimens, the common antigens that can diagnose and protect the human from amoebiasis is not fully known. Similarly, reports on exo-antigens in *E. histolytica* and their role in disease are not available. All these studies clearly suggest for an urgent detailed analysis of *E. histolytica* trophozoite antigens particularly, to those having both diagnostic and immuno-prophylactic potentials against amoebiasis. Preliminary results of antigenic analysis, using sera from immune or infected individuals, indicate that the whole amoebic extract, prepared from *E. histolytica* trophozoites, are highly complex and heterogeneous in nature. Studies on exo-antigens, clearly suggest that *E. histolytica* also releases certain antigen(s) in the extracellular milieu having similar diagnostic values as the conventional somatic antigens, which can be used for future immunodiagnostic studies against amoebiasis.

Somatic antigens of *E. histolytica*

Somatic antigen was prepared from 72-96 h old axenic trophozoites of *E. histolytica*. Briefly, trophozoites of axenic *E. histolytica* were dislodged from walls by chilling the tubes in ice bath followed by washing with PBS (pH 7.0). The counted trophozoites were sonicated for six 30 sec bursts at 20 kc and centrifuged at 10,000 x g for 30 min and the supernatant collected was termed as crude soluble antigen (CSA)(3). To determine the antigens of immunodiagnostic and immunoprophylactic importance in CSA, the crude antigen was analytically purified onto the Sephacryl S-300 column and four fractions were obtained (viz., FI to FIV respectively). Of those, FI and FII

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Author for correspondence

Dr. Pradeep Das, Asst. Director at above address.

contained mainly proteins whereas FIII contained polysaccharide moieties. These findings were once again confirmed by physical and immunochemical studies. The molecular mass of FI, FII, FIII and FIV was 660 kDa, 170 kDa, 65 kDa and 13 kDa, respectively(3).

The SDS-PAGE showed several discrete protein bands ranging from >200 kDa to 20 kDa in CSA. Similar banding patterns with lesser number of polypeptide subunits were observed in FI proteins. However, discrete polypeptides in the molecular mass of 180 kDa to 30 kDa and 140 kDa to 22 kDa were revealed with FII and FIII proteins respectively. Only few minor bands were seen with FIV protein(3).

By immunological analysis, FI antigen was found 8 times more antigenic than parent as well as other fractions both in CIEP and ELISA against the amoebiasis patients and immunized rabbit sera to whole trophozoites, thereby suggesting the use of this fraction in getting better results for immunodiagnosis of amoebiasis. The reaction of anti-FI antibody to live trophozoite of *E. histolytica*, clearly suggests its commonness with the surface antigens thereby suggesting the participation of these antigens in the host immune response.

The results of active immunization once again demonstrated the superiority of FI (91.6%) antigens over other fractions (FII and FIII 41.6% and 33.3%) and parent antigen (41.6%) in protecting the development of experimental hepatic amoebiasis. No protection was observed with FIV(3).

Monoclonal antibodies (Mabs) to FI antigens showed six clones which produced high titre anti-FI antibodies. The rationale for such an approach was due to the principle that (i) Mabs generated against specific epitopes, will help in development of a diagnostic test for amoebiasis cases directly from stool samples, (ii) to analyse all the Mabs for their specific role in the differentiation of pathogenic *E. histolytica* from non pathogenic *E. dispar* directly from the clinical specimens and (iii) to purify the antigens to the well characterized Mabs and assess their importance in immunoprophylaxis studies. Of the six, one Mab (NICED 11) which showed IgG1 isotype, high agglutination activity and reaction with single epitope present at 29 kDa

region of FI antigen was used in multi layer ELISA for the detection of amoebic antigen in stool specimen(4). The test could detect as low as 8 ng of amoebic antigen in faecal samples (equal to two trophozoites) and was found to be much more sensitive and specific than the previously described conventional methods. By further analysis, it was observed that this Mab has also the ability to differentiate between cases of active amoebiasis from asymptomatic cyst passers(4). This Mab is now routinely being used for direct diagnosis of pathogenic strains of *E. histolytica* in the stool samples.

Antigen specific to NICED 11 Mab was purified and characterized. Physicochemical analysis of affinity purified 29-kDa antigen revealed its glycoprotein nature and primary location on the surface of live trophozoites. Amphiphilic nature of this antigen was established in Triton X-114 phase separation studies and further confirmed by immunoblot assay. The reaction of purified 29-kDa antigen only with sera of symptomatic amoebiasis and not with asymptomatic cases in immunoblot assay clearly suggests the role played by this antigen in human amoebiasis and future use of this antigen in the differentiation of symptomatic amoebiasis to asymptomatic ones. Significant high levels of serum antibody titer ($P > 0.001$ than control) and cell mediated immune response against this antigen was observed in immunized animals. In protective efficacy analysis, the animals immunized with this antigen showed 72% protection against the development of hepatic amoebiasis(4). These findings clearly indicated the importance of this 29-kDa antigen for both immunodiagnosis and immunoprophylaxis against amoebiasis.

Excretory-Secretory Antigens of *E. histolytica*

It has been well demonstrated that cells of *E. histolytica* like many other organisms also release certain metabolites into the culture medium during their in vitro cultivation, called as excretory secretory antigen (ESA)(5). A comparative evaluation of ESA with somatic antigen in serodiagnosis of amoebiasis by ELISA, showed 100% specificity and sensitivity in cases of amoebic liver abscess and control samples by both the antigens. However, ESA was found slightly better than somatic antigens in the

serodiagnosis of acute amoebic dysentery and asymptomatic cyst-passers(6). But this requires further investigation with larger number of samples. The glycoprotein nature of ESA was established in protein-carbohydrate estimation and confirmed by physico-chemical analysis. SDS-PAGE protein profiles of ESA showed distinct coomassie blue bands in the range of 20 -200 kDa. Immunoreactive polypeptides of ESA to amoebiasis patient sera were observed at 170, 56 and 20 kDa, thereby suggesting the participation of these molecules in human amoebiasis. These preliminary results clearly suggest the diagnostic potential and important involvement of these antigens with the disease.

SUMMARY

Amoebiasis, caused by an enteric protozoan *Entamoeba histolytica*, is one of the major parasitic diseases of mankind. Current estimate suggests that the parasite infects about 10% of the world population at any given time. There is an urgent need to characterize the antigenic molecules of *E. histolytica*, and find out antigens which have both immunodiagnostic and prophylactic potential against amoebiasis. The results of somatic antigen analysis, using sera from immune or infected individuals, indicated that the whole *E. histolytica* trophozoites, are highly complex and heterogeneous in nature and both

immunodiagnostic and immuno prophylaxis activity remain mainly in a surface associated 29 kDa glycoprotein of *E. histolytica*. Future studies at molecular level particularly, genes responsible for expression of this protein, their homology with other proteins and structure analysis will give better understanding about this polypeptide. Studies on excretory secretory antigens, clearly demonstrated that *E. histolytica* like many organisms, also liberates certain antigenic moieties into the culture medium during in vitro cultivation and this antigen has similar diagnostic values like the conventional somatic antigens. It is important that the ESA should be prepared from the supernatant after collecting the cell and use for immunodiagnosis of amoebiasis. This is an additional source of antigen which will help in carrying out more tests using the same reagents. Further studies are needed to clarify the location of these molecules on the parasite, along with detailed biochemical and immunological characterization and their relation with the pathogenesis.

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