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Immunological characterization of axenic *Entamoeba histolytica* related antigens

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The amoebic antigen obtained from axenic culture of *E. histolytica* was fractionated by gel filtration on Sephadex G-200 column. Five different protein fractions (Fractions I to V) were obtained with molecular weights in the range of 12,500 to 2,83,000. Only fraction I was found to be homogeneous by polyacrylamide gel electrophoresis. The immunogenicity of these antigenic proteins, as evaluated by micro-ELISA system, showed that fraction I was the most antigenic in character. The results of ELISA also demonstrated the presence of common determinants on all the protein fractions. Fraction I can be considered as the amoebic antigen most suited for use in diagnostic assays.

There are several reports showing that amoebic antigen obtained from *Entamoeba histolytica* is a mixture of a number of component proteins with varying molecular weight[1-4]. The serological properties of the whole antigen have been extensively investigated[5,6] but very little is known about the exact composition and immunogenicity of its component proteins. It was considered that the use of the most antigenic and highly purified component protein was necessary to improve the sensitivity as well as specificity of the diagnostic assays for achieving efficiency and accuracy and for the purposes of immunisation to elicit high titre of antibody for developing acquired resistance in patients. The present study was undertaken to purify and characterize the various fractions of amoebic antigen prepared from *E. histolytica*, to determine the immunogenicity of each fraction and to find out the most immunogenic component to be used for serodiagnosis of amoebiasis.

Material & Methods

Antigen preparation and its fractionation : Amoebic antigen used in the present study was an axenic preparation of *E. histolytica*. NIH : 200 strain of *E. histolytica* was grown axenically in TPS-1 medium by the method of Diamond *et al*[7] and the protein content of the antigen was measured by the method of Lowry *et al*[8]. 0·2 ml of sonicated amoebic antigen (protein, 8 mg/ml) was chromatographed on Sephadex G-200 column (1·6×84 cm) and the elution was done

into 3 ml fractions with 0·05 *M* phosphate buffer containing 0·15 *M* NaCl (PBS), *p*H 7·2. The column was monitored for protein content at 280 nm in Beckman 25 spectrophotometer. Fractions corresponding to different peaks were pooled, concentrated and stored at —20°C till further use. The homogeneity of each fraction was tested by polyacrylamide gel electrophoresis (PAGE) using 7·5 per cent acrylamide gel in tris-glycine buffer, *p*H 8·3, at 4 mA per tube under non-dissociating conditions. The gel was stained with Coomassie brilliant blue.

Estimation of molecular weight : The molecular weight of individual fraction was determined as described by Irshad *et al*[9] using the formula :

$$Ve/Vo = 6{\cdot}984 - 1{\cdot}069 \text{ of log } M$$

where, Ve is the elution volume, Vo is the void volume and M is the molecular weight of the protein.

Evaluation of immunogenicity of antigenic fractions : The immunogenicity of each fraction was determined by an inhibition assay using pooled sera of ALA patients by ELISA technique of Gandhi *et al*[10].

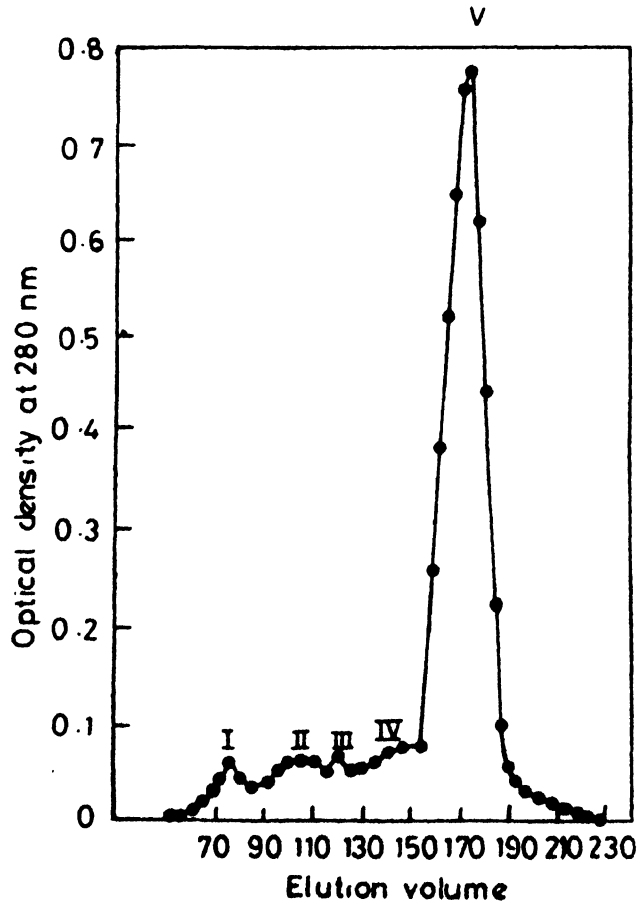

Fig. 1. Fractionation of axenic *Entamoeba histolytica* on sephadex G-200.

In brief, varying concentrations of each fraction were preincubated with fixed volume of known high titre pooled antibody positive serum for 18 h at 4°C followed by addition of these contents to the 2 wells of a microtitre plate precoated with whole antigen (5 μg/ml). The residual antibody in each sample was determined using horse radish peroxidase : protein-A conjugate (HRPO-Protein A). The immuno-reactivity was expressed as reduction in OD value at a fixed antigen concentration using OD value without antigen as control.

Results

A typical elution profile of amoebic antigen on Sephadex G-200 column is shown in Fig. 1. Five major fractions were obtained and their absorbance at 280 nm is shown. The molecular weights of these fractions were found to be 2,83,000, 1,08,000, 67,000, 31,000 and 12,500 respectively. Only fraction I was found to be homogeneous with a single band (Fig. 2) on polyacrylamide gel electrophoresis. The relative immunogenicity of these fractions as evaluated by inhibition ELISA showed that fraction I was most immunogenic as compared to other fractions including the whole antigen (Fig. 3).

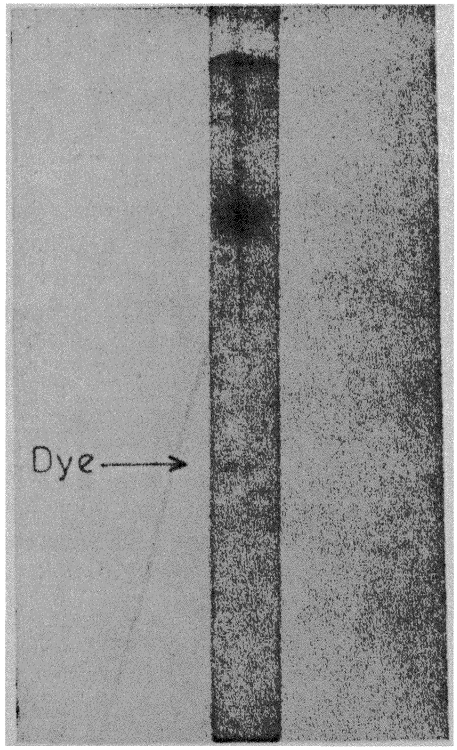

Fig. 2. Polyacrylamide gel electrophoresis (PAGE) of fraction I from Sephadex G-200. column.

Discussion

The amoebic antigen from *E. histolytica* is a complex mixture of several components of varying molecular sizes and net charges. There has been a considerable interest in the purification and characterization of these components but the complete elucidation of their structure has not yet been achieved. Attempts are being made to separate the individual components and characterize them for their immunogenicity. The results of our gel filtration studies have demonstrated that amoebic antigen consists of five different molecular fractions with varying molecular weight in the form of five distinct fractions. The molecular weight of these fractions were found to be in the range of 2,83,000 to 12,500. The findings of the present study are in agreement with those by Kettis *et al*[11] but different from those of Sawhney *et al*[12], who have shown only three fractions. The better resolution of amoebic antigen on long column of Sephadex G-200 could be a possible reason for our getting additional fractions.

Further analysis by PAGE showed that fraction I with high molecular weight of 2,83,000, was the only homogeneous

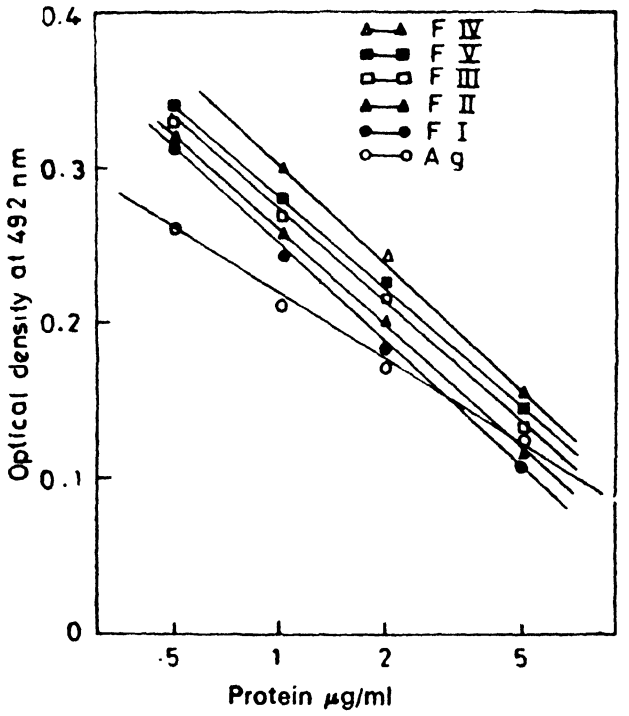

Fig. 3. Immuno-reactivity of different fractions (I to V) along with whole antigen as evaluated by inhibition ELISA.

fraction and gave only one band. The other four fractions were heterogeneous with multiple bands.

The results of relative immunogenicity of these antigenic proteins including the whole antigen, as evaluated by inhibition ELISA method, indicated that fraction I was the most immunogenic in character. This was assumed to be due to the high purity of this fraction. The presence of non-antigenic components in Fraction II to V, may interfere in antigen-antibody interaction and thus reduce the binding efficiency of antigenic fractions. Since fraction I is a high molecular weight protein its high immunogenicity may be attributed to a large number of determinants present on each molecule. Fraction I as such represents 6 per cent of the total amoebic antigen and may be easily purified in a single step gel-filtration. Being a large molecule, fraction I should prove to be the best antigen for active immunization.

In view of our observations, it may be concluded that fraction I obtained from single step gel filtration may prove to be the most suitable antigen for serodiagnosis of amoebic infection.

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