Evaluation of fractionated Wuchereria bancrofti microfilarial excretory-secretory antigens for diagnosis of bancroftian filariasis by enzyme linked immunosorbent assay

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Abstract. The Wuchereria bancrofti microfilarial excretory-secretory antigens were fractionated into ES1, ES2, ES3 and ES4 by ultra-membrane filtration and evaluated for their diagnostic utility by enzyme linked immunosorbent assay. Three of the four fractions showed antigenic activity (ES2, ES3 and ES4). The antigen fractions ES2 and ES4 were highly active in the detection of filarial IgM antibody in clinical filariasis and microfilaraemia respectively. The chemical characterization of the ES2 and ES4 antigen fractions showed that they were glycoproteins.

Keywords. Microfilarial excretory-secretory antigen; ultramembrane filtration; enzyme linked immunosorbent assay.

Introduction

Excretory-secretory (ES) antigens were highly sensitive and specific tools for the diagnosis of parasite diseases such as Chagas disease (Tarrant et al., 1965), toxocoriasis (de Savigny et al., 1977) and onchocerciasis (Schiller et al., 1980). Studies from this laboratory have shown that the Wuchereria bancrofti microfilarial (mf) ES antigens are highly sensitive in detecting antibody in filarial sera as well as in filter paper blood samples (Kharat et al., 1982; Malhotra et al., 1982). However mf ES antigen from culture fluid could not distinguish active infection from clinical filariasis in enzyme linked immunosorbent assay, using either mixed or specific anti-immunoglobulin-enzyme conjugates. Separation and characterization of different antigen fractions from the whole antigen has proved to be a meaningful approach in obtaining an active fraction with the required specificities (Sawada et al., 1969; Marcoullis et al., 1978; Kaliraj et al., 1982). This communication presents the fractionation and characterization of mf ES antigens and studies on their diagnostic utility for detecting human filariasis.

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Abbreviations used: ES, Excretory-secretory; ELISA, enzyme-linked immunosorbent assay; mf, microfilarial; PBS/T, 0·01 M Sodium phosphate buffer saline pH 7·2, containing 0·05% Tween 20; IgG+M+A, antihuman immunoglobulins; GMRT, geometric mean of the reciprocal of antibody titre.
Materials and methods

Microfilarial excretory-secretory antigens

The mf ES antigens were prepared as described by Kharat et al. (1982). W. bancrofti mf were separated from microfilaraemia blood samples by nucleopore membrane filtration and maintained for 15 days in medium 199 (3–4 thousand mf/ml medium) supplemented with organic acids and sugars of Grace’s medium (Paul, 1975). The medium was changed after every 24 h and the culture fluid was centrifuged at 13,000 g for 15 min. The supernatant was collected and the protein was estimated by Lowry’s method (Lowry et al., 1951). Then it was stored at –20°C until used, after addition of sodium azide to a final concentration of 0·1% as preservative.

Sera

Human sera (32 samples), belonging to different groups viz., normal subjects from endemic and non endemic regions (endemic and non endemic), filarial patients (microfilaraemia and clinical filariasis) were screened in this study. Filarial blood samples were collected from Sevagram and surrounding villages, which is an endemic area for nocturnally periodic form of W. bancrofti. Endemic normal samples were from healthy individuals living in Sevagram and its surrounding villages and having neither mf in their blood nor any clinical symptoms. The presence or absence of mf was confirmed by night blood (wet smear) examination. Non endemic normal blood samples were collected from students of this Institute coming from places like Chandigarh, Kashmir etc. where there is no filariasis immediately after their admission. Sera were separated from blood samples and stored at –20°C after addition of sodium azide as preservative.

Fractionation of mf ES antigen

The fractionation of mf ES antigen into different fractions by ultra membrane filtration was essentially as described by Nash et al. (1974) with S. mansoni homogenate and by Mok et al. (1977) with Histoplasma capsulatum antigen, except that Millipore ultrafiltration system (Millipore Corporation, Bedford, USA) was used in this study. One ml of culture fluid containing mf ES antigen was diluted to 20 ml with 0·05 M sodium phosphate buffer pH 7·2 and sequentially passed through CX-10 (NMWL 10,000), CX-30 (NMWL 30,000) immersible ultrafilters and PTHK 02510 (NMWL 100,000) pellicone ultra-filter membrane. At each stage of filtration, the filtrate was collected and the concentrate (0·1 ml) was diluted to 20 ml with the same buffer and refiltered. The process of dilution and refiltration was repeated twice and these filtrates were discarded. The concentrate was diluted to 20 ml with 0·05 M sodium phosphate buffer pH 7·2 for passing through the next filter in sequence. The filtrates collected first from CX-10, CX-30 and PTHK-02510 ultra filters were labelled as ES1, ES2 and ES3 fractions respectively and the concentrate on PTHK-02510 was diluted to 20 ml with 0·05 M sodium phosphate buffer pH 7·2 and labelled as ES4. All the four fractions were diluted 500 times with sodium carbonate buffer 0·06 M, pH 9·6 and used in indirect enzyme linked immunosorbent assay (ELISA) to assess their diagnostic utility.