

WUCHERERIA BANCROFTI : DETECTION OF MICROFILARIAE IN ASYMPTOMATIC MICROFILAREMIC INDIVIDUALS WITH SETARIA DIGITATA ANTIGENS

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Abstract. A dot-ELISA for detection of microfilariae of *Wuchereria bancrofti* in an endemic area was developed. This test can differentiate the endemic normals from the microfilaraemic asymptomatic individuals. Antigens of molecular weight 130 and 52 kDa of the cattle filaria worm *Setaria digitata* were used for this test. It was observed that these two antigens were also present in the serum of asymptomatic microfilaraemic individuals.

INTRODUCTION

Lymphatic filariasis is endemic over a wide geographic area covering tropical and sub-tropical Africa, Central and South America, Asia, and Oceania. In these zones, an estimated 751 million people are at risk of filariasis infection. Of the more than 78 million persons already afflicted, more than 90% are believed to harbor *Wuchereria bancrofti* (WHO, 1992). An important aim of filarial control programs is the elimination of microfilariae (mf) so that transmission can be reduced in an endemic area. Therefore the detection of microfilaraemic individuals is very important for the success of such programs. Night blood sampling is mandatory for the detection of mf in these individuals due to the nocturnal periodicity of the mf of *W. bancrofti*. Even the recently developed *W. bancrofti* specific DNA probes (Siridewa *et al*, 1994) or the polymerase chain reaction assay (Sirideva *et al*, 1994) require night blood collection. However, night blood filming is a problem in all endemic countries including Sri Lanka, due to the cost incurred, lack of compliance of the public and the lack of dedication of

the staff, particularly night blood filming staff.

The commercially available antigen detection assays for bancroftian filariasis, namely the antigen test kit based on monoclonal antibody Og4C3 (Trop-Ag *W. bancrofti*, JCU Tropical Biotechnology, Pty Ltd, Townsville, Queensland, Australia) and the ICT card test (ICT Diagnostics, Balgowlah, New South Wales, Australia) are very costly and therefore cannot be used for routine work including epidemiological surveys and control programs in Sri Lanka. In most filarial infections, microfilaraemia is associated with the absence of antibodies to microfilarial surface antigens (Dissanayake and Ismail, 1981), therefore the microfilaraemic, asymptomatic individuals cannot be detected by the presently available indirect fluorescent antibody test (IFAT), which can detect antibodies against the sheath of microfilariae.

These problems make it necessary for the development of more efficient, alternate, sensitive serodiagnostic methods which can be performed during day time using other alternative antigens, *eg* adult parasite antigens and circulating antigens. However the non-availability of adult *W. bancrofti* antigens has become an obstacle for the development of such serodiagnostic methods. Ottesen (1984) has reported that antigens from animal filariae can be used for the specific diagnosis of filariasis. Such studies have included the

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use of *Setaria digitata* antigens for the diagnosis of bancroftian filariasis (Dissanayake and Ismail, 1980).

Wuchereria bancrofti antigens have been isolated which have considerable similarity to the adult *Setaria digitata* antigens and an antigen SD 2-4 of isoelectric point around pH 3 was found to be useful in diagnosis of bancroftian filariasis (Dissanayake *et al*, 1982). A low molecular weight fraction (30 kDa.) of *S. digitata* was demonstrated to have allergenic activity in patients with lymphatic filariasis and endemic controls from Orissa, India (Beuria *et al*, 1995). A 29 kDa protein isolated from *S. digitata* was found to be of diagnostic value in patients having bancroftian filariasis (John *et al*, 1995). *S. digitata* surface antigens isolated by EDTA extraction and purified by affinity chromatography using *W. bancrofti* antibodies demonstrated about six (10-89 kDa) antigenic bands by immunoblotting (Theodore and Kaliraj, 1990).

Circulating immune complexes have been reported in *W. bancrofti* infections (Dissanayake *et al*, 1982). These antigens too may be a good source of antigens for diagnostic purposes because adults are not available for antigen extraction. Two monoclonal antibodies 13B4 and 15D6 have been developed against the antigenic epitopes, common between *S. cervi* and *Brugia malayi* and these are important in detecting circulating antigens in filarial infected individuals (Kaushal *et al*, 1994). Analysis in polyethylene glycol (PEG) precipitated immune complexes isolated from patients with bancroftian filariasis were found to be similar to the SD 24 antigen of *S. digitata* by SDS-PAGE autoradiography (Dissanayake *et al*, 1982).

The present study was carried out to isolate an antigen of *S. digitata* which may be used to develop a dot-ELISA for the diagnosis of microfilaremic, asymptomatic individuals.

MATERIALS AND METHODS

Preparation of antigens

All chemicals used were purchased from

Sigma Chemical Co (St. Louis, MO) and were of analytical grade. Adult *S. digitata* were collected from the peritoneal cavity of freshly slaughtered cattle at the abattoir, Peradeniya, Sri Lanka. The worms were transported in sterile saline to the laboratory, washed twice and stored at -80°C until used. For the preparation of antigens, the frozen adults were sliced using a sterile surgical blade and suspended in 10 mM Tris-HCl, (pH 7.4), 1 mM EDTA and 1 mM PMSF.

The mf of *S. digitata* were obtained from the same cattle that were used for collection of adults. The mf of *W. bancrofti* were collected from patients positive for mf in night blood filming.

The cuticle fractions (membrane fraction) of both adults and mf of *S. digitata* and mf of *W. bancrofti* were prepared according to procedures described by Cox *et al* (1981).

Patient populations

Sera were collected from 3 groups of individuals; *viz* (1) non endemic controls, (2) endemic controls and (3) asymptomatic microfilaremic individuals.

The non-endemic control group consisted of 12 pooled serum samples obtained from individuals living in Matale, Sri Lanka, which is non endemic for bancroftian filariasis and the endemic controls consisted of 60 pooled serum samples from individuals living in the endemic belt of Sri Lanka. The individuals of both groups were amicrofilaremic and were negative for the IFAT test.

SDS - PAGE and immunoblotting

Pooled sera from 60 microfilaremic, asymptomatic individuals obtained after examining 20 µl of night blood were grouped as asymptomatic microfilaremic individuals. These sera were negative for the IFAT test. SDS-PAGE was carried out using a Bio-Rad Mini Protean Slab cell. A 12% resolution and 4%, stacking gel was cast by the method described by Laemmli (1970). The membrane antigens were electrophoresed at 200 V for

approximately one hour.

The antigens were identified by immunoblotting with the serum samples of (1) non endemic controls, (11) endemic controls, (111) asymptomatic microfilaremic individuals, by the methods described by Towbin *et al* (1979). Identification of antigens of *W. bancrofti* from sera was carried out by using the method described by Weil and Fanya (1987). Sera from asymptomatic microfilaremic patients and non endemic controls were added to cyanogen bromide activated sepharose (CNBR) beads that were previously covalently linked to antibodies against *W. bancrofti* and incubated overnight at 4°C. These beads were washed thrice and the antigens were analyzed by SDS-PAGE and stained with silver stain.

Elution of proteins

The protein elution from SDS-gels was performed by grinding the gels containing the proteins in 0.05 M sodium phosphate buffer of pH 7.2 and leaving overnight at 4°C.

Dot ELISA

The dot-ELISA was carried out by spotting antigen solutions of varying protein concentrations on nitrocellulose paper and immobilizing at 37°C for 2 hours. These spots were then quenched with 2% bovine serum albumin, washed, exposed to different dilutions of sera from non endemic controls, endemic controls and asymptomatic microfilaremic individuals. These exposed spots were then treated with anti-human IgG peroxidase conjugate at 37°C for 10 minutes and were stained with 4-chloro naphthol to get visible reacting spots.

RESULTS

SDS gel electrophoresis of membrane extracts of *S. digitata* adults, *S. digitata* mf and *W. bancrofti* mf revealed approximately 17, 09 and 19 polypeptides respectively. Of these only two polypeptides of molecular weights 57 and 79 kDa were shared by the membrane extracts of all three.

Polypeptides of molecular weights 172, 130, 52, 45 and 24 were present only in the membrane extracts of the adult *S. digitata*.

SDS electrophoresis of the three membrane extracts and immunoblotting with sera of non endemic controls and asymptomatic microfilaremic individuals revealed that antigens of molecular weights 130 kDa and 52 kDa (adult *S. digitata* antigen) were recognized by the serum antibodies of asymptomatic microfilaremic individuals but these were not recognized by the serum of non endemic individuals (Fig 1). When individual sera of the symptomatic, microfilaremic group were subjected to this test it was revealed that 80% of the serum samples recognized the 130 kDa antigen and 52% of the serum samples recognized the 52 kDa antigen.

Serum of asymptomatic microfilaremic patients showed the presence of four antigens of molecular weights 24, 79, 52, 130 kDa (Fig 2). These were absent in the non endemic controls.

Dot-ELISA using the *S. digitata* adult antigens of molecular weights 130 kDa and 52 kDa gave a positive reaction with serum antibodies of asymptomatic microfilaremic individuals at a minimum dilution of 10 ng of protein per spot. Serum antibodies of the non endemic normal group gave a negative reaction and the endemic normal sera gave a positive reaction at a dilution of 40 ng per spot.

DISCUSSION

Antigens of molecular weights 52 and 130 kDa were recognized by serum antibodies present in microfilaremic asymptomatic individuals. But these were not recognized by sera from non endemic controls. The antigens of molecular weights 52 kDa and 130 kDa were present only in the adult stage of the cattle filaria worm *S. digitata*.

Further studies done by us revealed that these same antigens were present in the serum samples of asymptomatic microfilaremic in-

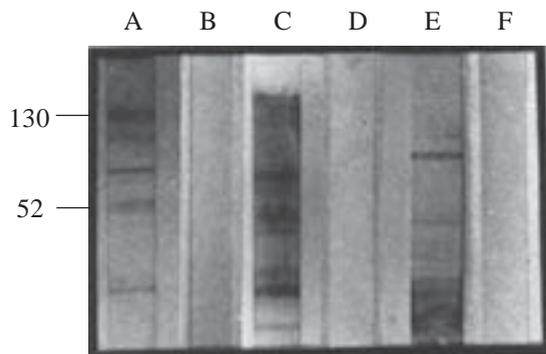


Fig 1—Membrane antigens of *S. digitata* and *W. bancrofti* were separated on SDS-PAGE, blotted onto nitrocellulose and exposed to sera obtained from microfilaremic asymptomatic patients and non endemic controls. Lane A (*S. digitata* adult antigen), Lane C (*S. digitata* mf antigen), Lane E (*W. bancrofti* mf antigen) exposed to sera from microfilaremic asymptomatic patients. Lane B (*S. digitata* adult antigen), Lane D (*S. digitata* mf antigen), Lane F (*W. bancrofti* mf antigen) exposed to sera from non endemic controls.

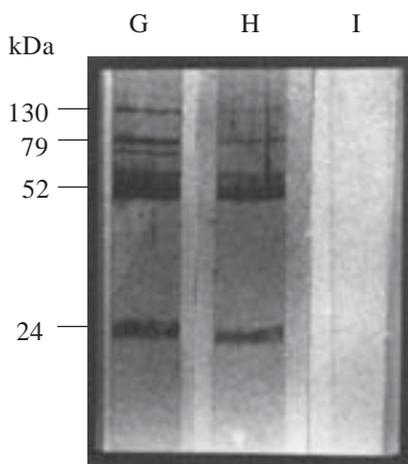


Fig 2—Cyanogen bromide activated sepharose (CNBR) beads covalently linked to antibodies against *W. bancrofti* were exposed to sera from microfilaremic asymptomatic patients and non endemic controls. These beads were subsequently subjected to SDS-PAGE and silver staining. Lane G,H exposed to sera from microfilaremic asymptomatic patients. Lane I exposed to sera from non endemic controls.

dividuals. Therefore it is highly likely that the antigens of molecular weights 52 and 130 kDa originate from adult *W. bancrofti*.

Similar studies carried out in Australia have led to the development of an ELISA test for diagnosis of bancroftian filariasis by raising monoclonal antibodies against antigens of molecular weights 50-60 kDa and 130 kDa. These monoclonal antibodies were raised against the antigens of the cattle filarial worm *Onchocerca gibsoni* which were located at the junction of the cuticle and the hypodermis of the adult worm, as well as in the mf (More and Copeman,1990).This may also suggest that these antigens are shared by a large group of filarial parasites and that they have a common ancestry.

We used the adult *S. digitata* antigen of molecular weight 130 kDa and 52 kDa to develop a dot-ELISA. Both antigens detected microfilaremic asymptomatic individuals at a concentration of 10 ng antigen, whereas the endemic normals gave a positive reaction at a very high concentration of 40 ng antigen per spot and the non-endemic normals gave a negative reaction.

These findings indicate that the microfilaremic asymptomatic individuals can be differentiated from endemic normals at a minimum concentration of 10 ng of *S. digitata* antigens of molecular weights 130 kDa or 52 kDa per spot by the dot-ELISA. The 130 kDa antigen was sufficiently sensitive to detect 80% of the persons classed as 'positive' by night blood film while the 52 kDa antigen detected only 52%. Since the cattle filaria worm *S. digitata* is readily available in large quantities, the dot-ELISA using antigens of 130 kDa may be of value in identification of microfilaremic asymptomatic individuals using day time blood samples at a reasonable cost.

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