IN VITRO RELEASED ANTIGENS IN DIAGNOSIS AND IMMUNOMONITORING OF FILARIA AND TUBERCULOSIS

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ABSTRACT

In vitro released antigens by living parasites or bacteria under *in vitro* maintenance or short term culture showing specific humoral immune response have been explored in development of immunodiagnosics for infectious diseases such as filariasis and tuberculosis in our laboratory. ELISA using *B. malayi* mf ES antigen has been explored for detecting IgG antibody by Indirect ELISA and antigen by Inhibition ELISA and in immunomonitoring of carriers as well as clinical filarial cases. A ten year follow up of mf carriers with DEC therapy showed disappearance of antigen and antibody followed by reappearance in few cases in an endemic area. None of the cases followed developed clinical symptoms suggesting the need for long term monitoring and treatment of microfilaraemic carriers. Further immunomonitoring was found to be useful in confirming filaria aetiology in the absence of microfilaremia and determining appropriate period of treatment of acute, early clinical and occult filarial infections for clinical relief and cure.

Indirect Stick Penicillinase ELISA system using Mtb EST-6 antigen for detecting tuberculous IgG antibody and a Sandwich Penicillinase ELISA system using affinity purified antibody for detecting circulating antigen were explored in tuberculosis. A combination of both the assay systems with a sensitivity of 70% and specificity of 98% was found to be promising in the precise diagnosis of pulmonary tuberculosis. Further antigen detection was found to be useful in bone and joint tuberculosis.

KEY WORDS : ES antigens, ELISA, Immunodiagnosics, Immunomonitoring filariasis, tuberculosis.

INTRODUCTION

The immunogenic products of the living organisms obtained through their *in vitro* maintenance, ambiguously known as 'metabolic antigens' are also called as 'exoantigens' or 'excretory-secretory' (ES) antigens. The ES antigens are expected to have potential advantages over antigens from other sources such as whole organism extracts or somatic antigens because of their simplicity, specificity and sensitivity. Further their continued release results in adjuvant effect to evoke good humoral immune response in the

host. In this laboratory ES antigens obtained by *in vitro* maintenance of microfilariae and tubercular bacilli have been explored extensively in the development of suitable immunoassays for filariasis and tuberculosis respectively.

BRUGIA MALAYI MICROFILARIAL (mf) ES ANTIGENS IN IMMUNODIAGNOSIS AND IMMUNOMONITORING OF BANCROFTIAN FILARIASIS

Due to the non-availability of sufficient parasite material from *Wuchereria bancrofti*, heterologous filarial antigens from closely related *Brugia malayi* have been explored for the immunodiagnosis of bancroftian filariasis. *B. malayi* mf obtained by peritoneal lavage of infected jirds,

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(*Mariones unguiculatus*) were maintained in RPMI 1640 medium (5000mf/ml) at 37°C for 48 h. Bm mf ES antigens were concentrated from the culture fluid by centrifugation followed by dialysis and ultramembrane filtration.

A) Detection of filarial IgG antibodies and circulating filarial antigen

Filter paper blood eluates or sera samples were analyzed for filarial IgG antibodies by indirect stick ELISA as described by Parkhe et al (1) using Bm mf ES antigen. Filarial antigen was detected by inhibition stick ELISA as described earlier (2) using IgG fraction of filarial serum immunoglobulins (FSIgG) coated onto CAM sticks and Bm mf ES antigen penicillinase conjugate.

Patients with clinical manifestations rarely show presence of filarial parasite in blood. Hence the demonstration of filarial parasite antigens or antibodies developed against them is useful in confirming filarial aetiology in such cases. Among acute filarial cases filarial IgG antibody positivity was 72%, 68% and 54% in generalized, cervical and inguinal lymphadenopathy cases respectively and only 28% in epididymo-orchitis cases. While filarial antigen positivity ranged between 43-67% in lymphadenopathy cases, as many as 70% of epididymo-orchitis cases were antigenaemic. Among the clinical filarial cases with chronic manifestations like lymphoedema, elephantiasis, hydrocoele, chyluria/haematuria, the positivity was high for filarial antibodies (67-84%) than for filarial antigen (29-58%). Screening of occult filarial cases showed 44-66% of sera of tropical pulmonary eosinophilia (TPE), mono or polyarthritis and central serous retinopathy to be positive for filarial antibodies. Filarial antigen assay picked up more number of TPE and tenosynovitis cases (61-62% positivity). Retroperitoneal lymphadenitis cases showed 66% and 80% positivity for filarial antibody and antigen respectively. The demonstration of filarial antibodies/antigens has been very helpful in indicating antifilarial therapy for successful management of these clinical conditions.

B) Immunomonitoring of microfilaraemic, acute, chronic and occult clinical filarial cases during DEC therapy

Chemotherapy remains the main strategy in

the reduction of filarial transmission and morbidity. Over the last five decades diethylcarbamazine citrate (DEC) has been the drug of choice in the treatment of lymphatic filariasis. It is effective, safe and relatively cheap. Various regimens of DEC have been administered by different workers over the years. It has now been realised that a single dose or single course of DEC is not sufficient to clear all the adult filarial worms and hence long term and repeated treatment is necessary for effective cure. The inability to detect and quantitate lymphatic dwelling adult parasites and the limitations of night blood smear examination for mf in chronic and occult stages made it difficult to effectively follow up the filarial cases during treatment with DEC. Hence changes in the immunological parameters following DEC therapy have been explored as evidences for elimination of infection with simultaneous clinical improvement in the affected people.

A ten year follow up study was done on 27 microfilaraemic cases during treatment with DEC to understand immune status and recurrence of infection if any (3). Of the 27 mf carriers, 13 cases (Group A) never showed reappearance of mf in the peripheral blood after DEC therapy. All of the remaining 14 cases (Group B) showed reappearance of mf on one occasion or the other within 2 years period and 2 of 10 cases examined in this group showed mf positivity at the end of 10th year. While all the 13 cases in Group A became negative for filarial antibodies and antigen by the end of year 4, 22 & 50% of them turned positive again for antigen and antibodies respectively at the end of 10th year. In Group B cases, while all the 13 cases turned amicrofilaraemic by 60th day, 11 of them were still positive for filarial antigen and all the 13 showed the presence of filarial antibody. The fact that 12 of them became microfilaraemic again by the end of 1 year suggests that the detection of filarial antigen and antibody is more reliable indicator than demonstration of mf for the presence of active infection. At the end of tenth year, 50% and 70% of these cases (Group B) showed the presence of filarial antigens and antibody respectively. It is of interest to note the varying response to DEC in individuals stressing the necessity for long term monitoring of the infected cases during treatment. The reappearance of filarial antigen and antibody in a significant number of microfilaraemic cases in both the groups indicates the existence of

infection possibly due to reexposure to the parasites in the endemic areas. However none of the 27 cases followed up with chemotherapy in this study developed any clinical manifestations(3). This study demonstrated that the detection of antigen or antibody can be used as immunological marker for assessing disease status.

In another study from this laboratory(3) a total of 130 cases of acute, chronic and occult clinical filariasis were treated with repeated doses of DEC (6 mg /kg body weight per day for 21 days in a month) for 3 to 12 months and followed up for one year. During the follow up, the patients were examined clinically after every 3 months and blood samples were collected for determination of filarial antibody and antigen levels. The antigen / antibody positive cases were continued further with DEC treatment. DEC treatment was stopped as and when the patients turned negative for both filarial antibody and antigen associated with clinical relief and cure.

The long term DEC treatment resulted in a regular decrease in both antibody and antigen

levels in all the followed up cases along with clinical improvement. Except for 8% of chronic cases, all the other followed up cases turned negative for both antigen and antibody by the end of one year. Associated with sero conversion, acute, grade I chronic and occult filarial cases showed marked clinical improvement with reversal of symptoms in majority of the cases. Among the grade II and grade III chronic cases, the seroconversion was found to be associated with either reduction of manifestations to a minimal level or prevention of further worsening indicating the cure of active infection in such cases. The DEC treatment varied from 3-12 months in acute, chronic and occult filarial cases emphasizing the need of immunomonitoring such cases for determining the period of treatment for successful management (Table 1). Since microfilariae can not be detected in the peripheral circulation in all such clinical cases and adult worm demonstration is not easily possible, the presence of antibody or antigen could be taken as evidence of active infection during therapy.

Table 1 : Duration of DEC treatment in different clinical presentations of Filariasis based on the results of Immunomonitoring

Clinical Group	No. of Cases	DEC treatment	
		Duration (Months)	Percent of Cases Covered
Acute clinical filariasis			
Funiculitis, epididymo-orchitis (acute), minimal	22	3	36%
Hydrocele (acute), ADL (Acute attack), lymphoedema (<45 days duration with and without pain).		6	55%
		12	9%
Chronic clinical filariasis			
Hydrocele (grade I, II & III) & elephantoid cases, lymphoedema (I, II & III) and elephantiasis cases, chronic ADL, chronic epididymo-orchitis and fibroadenoma breast	62	3	16%
		6	61%
		12	23%
Occult filariasis			
TPE & monoarthritis	5	3	20%
		6	80%

In our institute's hospital for the last 7 years, routine immunomonitoring of clinical filarial cases is being done based on antigen and antibody detection and long term DEC therapy (6 mg/ kg body wt for 21 days in a month as long as 3 to 12 months) showed considerable relief and cure in early and atypical clinical manifestations such as asthmatic bronchitis, monoarthritis, pulmonary eosinophilia and pneumonia in children and minimal hydrocele, epididymo-orchitis, lymphangitis, lymphadenitis, pain and swelling in limbs and joints in adults, living in filarial endemic areas.

MYCOBACTERIUM TUBERCULOSIS H37Ra EXCRETORY - SECRETORY ANTIGENS IN DETECTION OF PULMONARY AND EXTRA-PULMONARY TUBERCULOSIS

Over the years various mycobacterial antigens ranging from crude (culture filtrate, whole cell sonicate, PPD) to purified antigens (Antigen-5, glycolipid antigens, lipoarabinomannan) have been explored in the immunodiagnosis of tuberculosis. The secreted antigens have drawn special attention of many workers for the fact that these antigens are believed to be more or less

similar to the in vivo released mycobacterial antigens.

Mycobacterium tuberculosis H37 Ra excretory-secretory (ES) antigen was prepared by *in vitro* culture of the bacilli in synthetic Souton medium as described earlier (4). M.tb ES antigen was then partially purified by 6% trichloroacetic acid precipitation. The TCA supernatant fraction (M.tb EST antigen) was further fractionated by SDS-PAGE to obtain an active M.tb EST-6 antigen with molecular weight range of 37-45 kDa. M.tb EST-6 antigen was used in stick Indirect ELISA to detect tuberculous IgG antibodies.

For the detection of circulating tuberculous antigen, Sandwich ELISA was developed using affinity purified goat anti EST antigen antibodies.

The results of analysis of 128 sera for tuberculous IgG antibodies and circulating tubercular antigen are summarized in Table 2. While the antibodies were detected with a sensitivity and specificity of 83% and 91% , the antigen assay showed a sensitivity and specificity of 79%. Using a combination of both the assays for antibody and antigen it was possible to detect pulmonary tuberculosis cases with a sensitivity of 70% and specificity of 98%.(Table 2)

TABLE 2 : Comparative analysis of antibody and antigen positivity in pulmonary tuberculosis.

Group	Total number screened	Number (%) positivity for		
		Tuberculous IgG antibody*	Tubercular antigen**	Both antibody & antigen
A.Pulmonary TB (smear positive)	58	49(84%)	48(83%)	44(76%)
B.Pulmonary TB (Clinically suspected & ATT responded)	13	10(77%)	08(61%)	06(46%)
C.Disease control	28	03(11%)	05(18%)	- (-)
D.Healthy control	29	02(7%)	07(24%)	01(3%)

* The serum dilution at 1:600 was taken as the threshold level for positivity.

** The serum dilution at 1:100 was taken as the threshold level for positivity.

M.tb EST antigen was purified on DEAE ion exchange column chromatography to obtain an active antigen fraction EST-DE1. Stick direct ELISA using EST-DE1 antigen and Sandwich ELISA using affinity purified goat anti EST antigen antibodies were used for detection of tuberculous IgG antibodies and antigen respectively in bone and joint tuberculosis.

In osteoarticular tuberculosis antibody detection showed a sensitivity of 83% and specificity of 75%, whereas antigen detection assay showed a sensitivity of 75% and specificity of 91%. Thus antigen detection assay was found to be better

than antibody assay in extra-pulmonary tuberculosis.

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