EVALUATION OF RECOMBINANT CHITINASE ANTIGEN IN SEROLOGICAL DIAGNOSIS AND SURVEILLANCE OF LYMPHATIC FILARIASIS

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Abstract. Apply recombinant chitinase fusion protein antigen, enzyme-linked immunosorbent assays examined anti-filarial antibodies and evaluated of useful value in serological diagnosis and surveillance of lymphatic filariasis. The test jirds were immunized and infected by chitinase and *B. malayi* third stage larvae respectively. Functional protein molecular of chitinase was analyzed by SDS-PAGE, Western blot. The result shown that jirds from microfilaremia (mf) and donors with Mf were directly to react with chitinase antigen that positive rate was 100%, but Mf-xt antigen was only 80%. Normal jirds and persons sera from unepidemic control donors all were negative. False positives of 5% and 20% reacted with chitinase and Mf-xt antigens respectively. The results indicate that recombinant chitinase antigen is suitable for detection of active occult or patent lymphatic filariasis with daytime blood samples in residents of endemic areas, is easy to be performed and inexpensive.

INTRODUCTION

Filarial nematodes such as *Brugia malayi* and *Wuchereria bancrofti* infect nearly 80 million people worldwide and are major causes of morbidity and economic loss in endemic areas (WHO, 1992).

Lymphatic filariasis transmission has been basic control in China. At present, one of all detection of new infections are very important measure for eradicate filariasis in endemic area. Optimal parasitologic diagnosis requires examination of blood samples collected at night because of the characteristic nocturnal periodicity of microfilaremia resulting from infection with lymphatic filarasis. Due to long-term filariasis control program was carried out including large-scale blood examination and chemotherapy with DEC, microfilariae density are lower in the peripheral circulation.

Traditional serodiagnostic techniques based on detection of antibodies to either crude filarial extracts or fractions generally are not specific enough to be useful as diagnostic tests.

Recent studies indicate that gene recombinant filariae antigens provide a possible approach to interrupt transmission by reducing the number of microfilariae in the blood of the human population or by interfering with parasite molecules that appear to be necessary for the penetration of the mosquito midgut and the subsequent development of microfilariae in the insect. Some of these shortcomings can be overcome by the use of recombinant antigens.

The purpose of the present study was evaluation of rec-chitinase antigen using serological diagnosis and surveillance of lymphatic filariasis.

MATERIALS AND METHODS

Rec-chitinase filariae antigen: Rec-chitinase antigen used in this study was a gift from Dr Piessens, Harvard School of Public Health, Boston. MA, USA.

Mf-solube extract (Mf-xt) antigen: Microfilariae of *B. malayi* were harvested from peritoneal cavities of infected jirds. Microfilariae (Mf) were washed extensively in sterile phosphate-buffered saline (PBS), freed from contaminating host cells by centrifugation over a ficoll gradient, sonicated and extracted at 4°C with constant agaitation in PBS containing I mm EDTA. The soluble material was removed by centrifugation, and the resulting supernatant was stored at -70°C until used. The protein content of parasite extracts and preparation of recombinant antigens was determined by the method of Bradford (1996).

Samples of sera: (l) Group of 20 microfilaremia jird, normal jird sera were prepared from blood of the retroorbital plexus and stored at -20°C until tested. (2) Group of 20 microfilaremia patients, amicrofilaremia from De Jiang county filaria epidemic area of Guizhou Province and unedemic area from middle school students of Guiyang city. Sera were prepared from blood of venous respectively, and stored at -20°C.

Preparation anti-rec-chitinase antigen antibody: Groups of 10 male jirds, 6-8 weeks old were immunized by IP injection with 10μg/each animal of chitinase or MBP protein emulsified in Freund's complete adjuvant and give a booster immunization with antigen in Freund's incomplete adjuvant 3-5 times, measure antibody level, sera were separated from blood and stored at -20°C.

Recognition of rec-chitinase protein: Rec-chitinase antigens protein molecules were recognized by SDS-PAGE, Western blotting with antibody reaction from jirds by the immunized chitinase (Towbin *et al*, 1979).

Serologic assays: Enzyme-linked immunosorbent assays (ELISA) with the recombinant fusion protein chitinase were done. Briefly, wells of microtiter plates were coated with 10μg/ml of chitinase or control maltose binding protein in carbonate buffer (pH9.6) at room temperature (R/T) for 1 hour or overnight at 4°C. The plates were blocked with PBS containing 0.5% Tween 20 for 30 minutes at R/T. Test sera were diluted 1:100 in PBS contain 5% normal goat serum and allowed to react at 1 hour R/T. The plates were then washed three times in PBS-Tween 20 and the bound antibodies was detected with peroxidase-conjugated goat anti-human IgG and 3.3′ 5.5′ tetramethylbenzidine substrate. The reaction was stopped with 50μl of 2N H_2SO_4 and quantified spectrophotometrically at 450 nm. Positive and negative reference sera were included in each assay. All sera were tested induplicate against chitinase and control MBP. Results are expressed as net optical density (OD) reading (average OD with chitinase minus average OD with MBP alone). Readings 0.1 were considered positive.

RESULTS

Recognized of chitinase protein molecule by jirds anti-chitinase antibody: Pre-and post immunization sera were prepared from blood of jirds by immunized with rec-chitinase antigen and MBP respectively. Antichitinase antibody were measured by ELISA and Western blotting with chitinase, Mf-xt and MBP antigens respectively (Wang *et al*, 1997; Juliet *et al*, 1992; Dissanayake S *et al*, 1992; Fuhrman and Piessens, 1989). Immunization with chitinase but n pwith MBP, elicited antibodies to native Mf chitinase. On Western blotting, jird antichitinase antibodies identify one specific filaria antigen of estimated molecular weight 69kDa bearing the epitope to which this antibody reacts. This antigen is present in extracts of Mf, but it is absent from similar extracts of infective larvae (L_3) and adult worms of *B. malayi.*

Preferential recognition of rec-chitinase antigen by jirds sera from microfilaremic subjects: Testing microfilaremic jird sera directly reacted with recombinant chitinase antigen (100%) but not with MBP. Normal jird sera were all the negative by chitinase antigen and MBP assay.

Preferential recognition of rec-chitinase antigen by patients sera from donors with microfilaremia of *W. bancrofti* and *B. malayi*: The results (Table 1) clearly indicate that rec-chitinase antigen is preferentilly recognized by sera from microfilaremic donors with *W. bancrofti* and *B. malayi*. In addition, 80% (16/20) of seropositivity with rec-chitinase antigen in amicrofilaremic persons reflects the presence of active filarial infection.

The prevalence of antibodies to microfilarea-extracts in jirds and human sera subject by ELISA: In the microfilaremia jirds the positivity rates were 80%, normal jirds were negative. Microfilaremic donors seropositivity rates were also 80%. 20% sera from endemic control residents in whom no microfilaria were detected by filtration of 1 ml of venous blood reacted with Mf-xt, and 5% sera from unepidemic control donors presented false sera positivity. Overall results of sera detected are summarized in Table 1.

DISCUSSION

The present study has shown that in microfilaremic jirds 100% have antibody to rec-chitinase protein antigen.

We did not detect antifilaria antibody from normal human sera of students from non-endemic areas. Amicrofilaremia of epidemic area donors antibody positive rate was 5%. This data was compared with the results by mf-xt antigens that chitinase antigen was more sensitive and specific reproducible than Mf-xt to determine antifilaria antibody.

The present study was performed to determine whether recombinant fusion protein antigens would be a useful antigen for serological diagnosis and surveillance of filariasis and this diagnostic test based on rec-chitinase antigen whether would be widely applicable in geographically distinct filariasis-endemic areas. Our preliminary results indicate that overall response rate to this one recombinant antigen is 100% in individuals and jirds with patent filariae. We therefore conclude that rec-chitinase is suitable for detection of active occult or patent lym-

Table 1
Comparison of anti-filaria antibodies in sera from Jirds and human with rec-chitinase and Mf-xt antigens detection.

Serum sample group (No)	Antigens	The secra antibody levels to chitinase by ELISA		The sera antibody levels to MBP by ELISA	
		Positive(%)	Antibody titer OD($\bar{X}\pm S\bar{X}$)	Positive(%)	Antibody titer OD($\bar{X}\pm S\bar{X}$)
Microfilaremia of Jirds (20)	Rec-chitinase	20 (100)	0.228±0.014	0	0.022±0.002
	Mf-xt	16 (80)	0.187±0.015	-	-
Normal of jirds (20)	Rec-chitinase	0	0.021±0.0037	0	0.012±0.001
	Mf-xt	0	0.0195±0.0023	-	-
Microfilaremia of Patients (20)	Rec-chitinase	20 (100)	0.351±0.0079	0	0.023±0.003
	Mf-xt	16 (80)	0.139±0.0110	-	-
Endemic controls (20)	Rec-chitinase	1 (5)	0.051±0.0054	0	0.02±0.0015
	Mf-xt	4 (20)	0.085±0.0138	-	-
Non-endemic control (20)	Rec-chitinase	0	0.024±0.0035	0	0.011±0.022
	Mf-xt	1 (5)	0.029±0.006	-	-

Mf-xt: Microfilarial extract

phatic filariasis with daytime blood samples.

Although anti-rec-chitinase antibody responses are associated with the microfilaramic state, a lack of correlation between antibody titers and parasitemia levels is to be expected, *ie*, it is likely that an antibody response to rec-chitinase indicates the Mf-density high or low in blood.

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