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POTENTIAL USE OF IgG2-ELISA IN THE DIAGNOSIS OF CHRONIC ELEPHANTIASIS AND IgG4-ELISA IN THE FOLLOW-UP OF MICROFILARAEMIC PATIENTS INFECTED WITH *BRUGIA MALAYI*

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SUMMARY Sera from fifty subjects with different presentations of Brugian filariasis and from common soil-transmitted helminth infections were tested for specific anti-filarial IgG and its subclasses. Anti-filarial IgG, IgG1 and IgG3 showed cross-reactivities with soil-transmitted helminthic infections and no significant differences in optical densities among the various groups of filarial patients. In comparison with other groups of subjects, IgG4-ELISA of sera from microfilaraemic patients and some previously microfilaraemic patients showed a significant increase in optical density readings, while IgG2-ELISA showed elevated optical density readings in sera of patients with chronic elephantiasis. Therefore IgG2-ELISA is potentially useful in the diagnosis of brugian chronic elephantiasis while IgG4-ELISA may be beneficial for follow-up diagnosis of treated microfilaraemic patients.

Lymphatic filariasis caused by *Brugia malayi* and *Wuchereria bancrofti* affects an estimated 80 million people world-wide (1). The disease has wide geographical distribution but is most prevalent in Africa and Asia. The species *Brugia malayi* however is predominantly found in Southeast Asian countries.

The diagnosis of lymphatic filariasis have been a problem due to the spectrum of clinical presentations and the lack of a specific and sensitive test. There have been developments in the diagnosis of microfilaraemic patients by Ottessen *et al.* (2) and Lal & Ottessen (3) who reported the prominence of anti-filarial IgG4 in this category of patients infected with *Wuchereria bancrofti;* and the DNA assay developed by Poole and Williams (4). In this study we investigated the immunodiagnostic potential of anti-filarial IgG subclasses in the differential diagnosis of filariasis due to *Brugia malayi*.

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MATERIALS AND METHODS

Sera

Sera from 70 subjects were taken in the daytime i.e. 21 endemic normals (NS I; asymptomatic amicrofilaraemics in endemic area), 10 non-endemic normals (NS II; city-dwellers), 6 microfilaria positive patients (MF), 6 previously confirmed microfilaria positive patients who had received treatment (PMF); 7 chronic elephantiasis patients (CE); 5 *Ascaris lumbricoides* positive (AS); 5 *Trichuris trichiura* positive (TR); 5 hookworm positive (HW) and 5 mixed infection with *Ascaris, Trichuris* and hookworm (MX).

Antigen preparation

Meriones unguiculatus (jirds) were infected intraperitoneally with third stage larvae of *Brugia malayi* to obtain adult worms. The antigen was prepared according to the previously described procedure (5). Briefly, the adult worms were sectioned with a scissors, ground in a Potter's homogenizer in phosphate buffered saline, pH 7.4 (PBS) and then sonicated. The preparation was then centrifuged at 2 000 g for 5 minutes and the resulting supernatant was used as the crude soluble antigen of *Brugia malayi*. The protein content was determined by the Bio Rad protein assay; the antigen was then aliquoted and stored at -70 ^{0}C.

ELISA

The procedure for the ELISA was performed as previously described with some modifications (6). Briefly 10 ug/ml *B. malayi* antigen was coated at 4^{0}C overnignt in polyvinyl microtiter plates (Falcon, USA). The plates were then washed with PBS containing 0.05% Tween 20 followed by the addition of 0.5% bovine serum albumin in PBS. Serum specimens at 1: 50 dilution were then added and incubated for 2h at room temperature. After a washing step, 1:1000 dilution of the anti-IgG1/IgG2/IgG3/IgG4 conjugated to digoxigenin (DIG; Boehringer Mannheim, Germany) were incubated for 2h. This was followed by incubation with anti-DIG-peroxidase at 1: 1000 for a further two hours before the addition of the chromogenic substrate.

In the detection of anti-filarial IgG antibody, indirect ELISA was performed using 1:800 dilution of anti-human IgG conjugated to peroxidase.

RESULTS

Figure 1 shows the results of the ELISA in the detection of anti-filarial IgG antibody in the various groups of subjects. High optical densities readings (O.D.) were observed in filaria patients as compared to normal subjects, however sera from soil-transmitted helminth infected patients also gave high O.D. values. Therefore determination of anti-filarial IgG antibody alone is of no diagnostic significance especially in underdeveloped

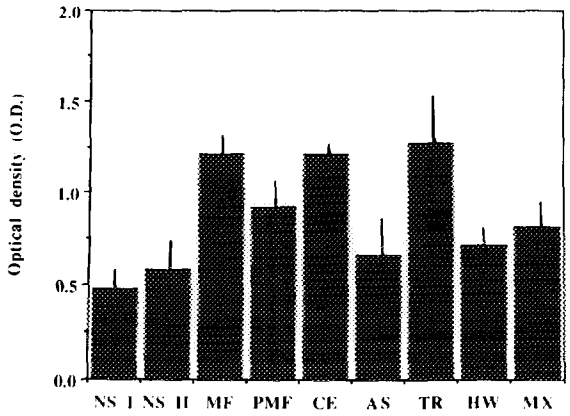

Figure 1. IgG-ELISA. Cross-reactivities are observed between filarial patients and patients with soil-transmitted helminthiases. NS I: endemic normals; NS II: non-endemic normals; MF: microfilariae positive; PMF: previously microfilriae positive (i.e. treated); CE: chronic elephantiasis; AS: *Ascaris lumbricoides* positive; TR: *Trichuris trichiura* positive; HW: hookworm positive; MX: mixed infection with *Ascaris, Trichuris* and hookworm.

areas where polyparasitism is common. Figure 2 shows the results of anti-filarial IgG1 and IgG3 antibodies; no particular differential pattern in the humoral responses were observed. The results of the IgG2-ELISA and IgG4-ELISA in the different groups of subjects are shown in Figure 3. In the IgG2-ELISA significantly elevated O.D. readings were shown in chronic elephantiasis patients (CE) as compared to the other groups of subjects. In the IgG4-ELISA, sera from microfilaraemic patients (MF) and three previously microfilaraemic patients (PMF II) showed very high O. D. values as compared to the other subjects. The other three previously microfilaraemic patients

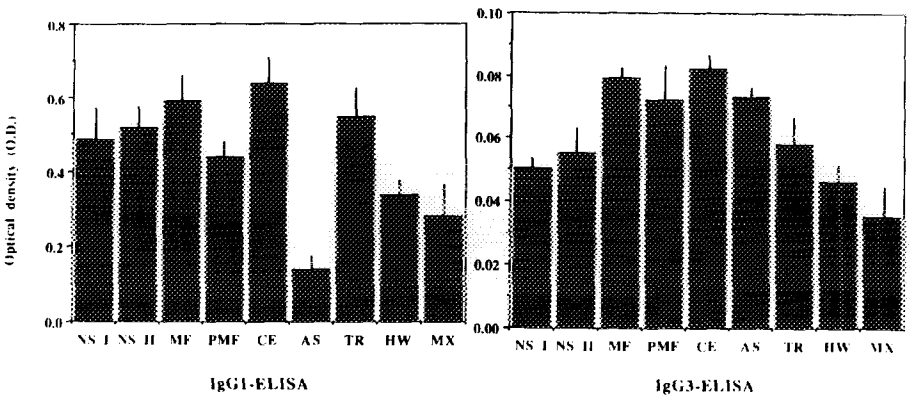

Figure 2. IgG1-ELISA and IgG3-ELISA. The optical densities of the various groups of subjects demonstrated that measurement of these two subclasses of IgG antibodies are not specific to diagnose filariaisis. NS I: endemic normals; NS II: non-endemic normals; MF: microfilariae positive; PMF: previously microfilriae positive (i.e. treated); CE: chronic elephantiasis; AS: *Ascaris lumbricoides* positive; TR: *Trichuris trichiura* positive; HW: hookworm positive; MX: mixed infection with *Ascaris, Trichuris* and hookworm.

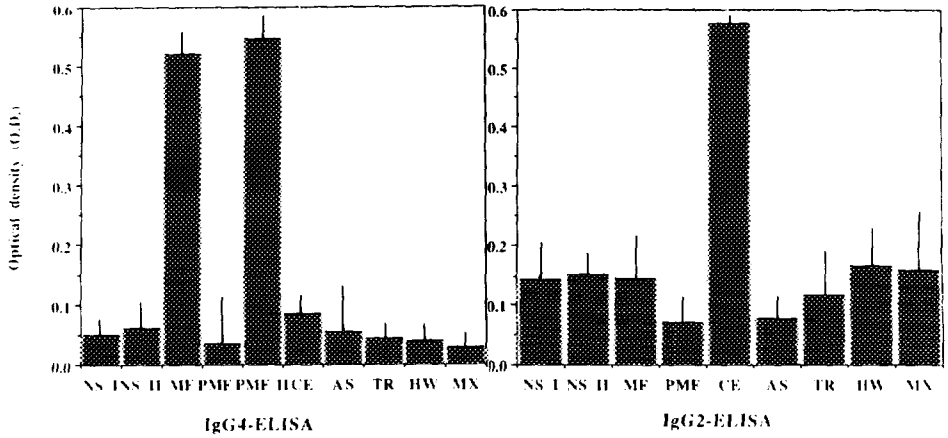

Figure 3. IgG2-ELISA and IgG4-ELISA. The results showed that IgG4-ELISA gave high optical density readings in microfilaraemic patients (MF) and some previously microfilaraemic patients (PMF II). IgG2-ELISA showed significant elevated O.D. values in chronic elephantiasis patients. NS I: endemic normals; NS II: non-endemic normals; MF: microfilariae positive; PMF and PMF II: previously microfilriae positive (i.e. treated); CE: chronic elephantiasis; AS: *Ascaris lumbricoides* positive; TR: *Trichuris trichiura* positive; HW: hookworm positive; MX: mixed infection with *Ascaris, Trichuris* and hookworm.

(PMF) demonstrated low O.D.values. Therefore these results demonstrated the immunodiagnostic potential of IgG4 as a marker of treatment effectiveness and IgG2 as a marker of chronic elephantiasis in *B. malayi* infection.

DISCUSSION

Previous studies have demonstrated high levels of anti-*B. malayi* IgG antibodies in patients infected with *Wuchereria bancrofti* (2, 7). This study on *B. malayi* infected patients showed a similar pattern. However since high levels of anti-filarial IgG antibodies were also observed in soil-transmitted helminthic infections, the detection of IgG alone is not specific for diagnosis of filariasis.

The DIG-ELISA demonstrated an enhanced anti-filarial IgG2 antibody response in patients with chronic elephantiasis due to *Brugia malayi*. In the study done by Ottessen *et al.*(2), they reported that anti-filarial IgG 4 and IgG1 responses (and not IgG2) were elevated in patients with chronic elephantiasis due to *Wucheria bancrofti*. Differences exists between *Brugia malayi* and *Wuchereria bancrofti* in their morphology, disease manifestations, animal susceptibility and antigenic components (1, 8); therefore differences in the anti-filarial IgG-2 responses between patients with these two kinds of infections may be expected. Phosphocholine (PC) is an immunodominant cross-reactive determinat that evokes a vigorous immune responses in bacterial, fungal, protozoan and helminth infections (9, 10, 11). The IgG antibody response to PC in normal humans is predominantly of the IgG2 subclass (3); therefore experiments will

be performed to preabsorb the chronic elephantiasis sera with PC and measure the levels of PC-specific IgG2 antibodies in the different groups of patients.

Investigators who studied*Wuchereria bancrofti* infections, (2, 3) reported that IgG4 displayed enhanced diagnostic specificity in patients with microfilaraemia and tropical pulmonary eosinophilia. Our study using *Brugia malayi* patients also demonstrated the same phenomena in microfilaraemic individuals. In addition we studied patients who were previously confirmed to be microfilaraemic and were treated with diethylcarbamazine. Out of the six patients, three gave low optical density values while the other three showed optical densities as high as the microfilaraemic patients although their daytime blood specimens for microfilaria gave negative results by both Knott's concentration and membrane filtration. The latter group of patients may thus have had inadequate treatment or low drug compliance due to the side effects of diethylcarbamazine (DEC). The microfilariae concentration techniques will be repeated on their midnight blood samples to detect low levels of microfilaraemia and a second course of DEC will be administered to monitor changes in their anti-filarial IgG4 antibody levels.

Although microfilaraemic patients do not show clinical disease, studies have shown that these asymptomatic microfilaraemics have renal abnormalities, hematuria, proteinuria, abnormal lymphatics characterised by dilatation of lymphatics, bizarre lymphatic channels and existance of collateral lymphatics as demonstrated by lymphoscintigraphy and ultrasound. They are also immunosuppressed and hyporesponsive to parasite antigens (1). Therefore it is important that proper follow-up of microflaraemic patients be undertaken to arrest the development of lymphatic pathology.

In conclusion this study documented the potential use of anti-filarial IgG2 antibody in the diagnosis of brugian chronic elephantiasis and the use of anti-filarial IgG4 antibody in the follow-up of microfilaraemics infected with *Brugia malayi*. In addtion to the immunodiagnostic potential of the above observations, these findings may also be useful in the understanding of the immunopathogenesis of brugian lymphatic filariasis.

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