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Anti-filarial IgG Antibodies in Patients with Bancroftian Filariasis and Tropical Pulmonary Eosinophilia

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Anti-filarial (anti- *B. malayi* adult as well as anti- *B. malayi* microfilarial) IgG antibody levels were measured by enzyme linked immunosorbent assay (ELISA) in asymptomatic microfilaria carriers, acute, chronic and tropical pulmonary eosinophilia (TPE) patients, endemic and non-endemic controls. Controls from endemic areas had higher antibody titres compared with controls from non-endemic areas. The antibody response in different groups of filariasis patients was not stage specific. There were no association between clinical disease and antibody levels except in TPE. Though TPE patients had very high antibody levels, a proportion of them had low levels suggesting heterogeneity in TPE population.

Most of the attention on filariasis immunity has been focussed around the humoral antibody response[1]. Almost every available assay has been employed to measure antibody response in filariasis patients[2,3]. Often, claims have been made about the specificity of the assays and their use in diagnosis of filarial disease[4]. Some correlations have been established between the microfilaraemia and the antibody levels[5]. However, there is a need for studies to correlate the antibody response, qualitatively and quantitatively, with clinical progression and manifestation of the disease. We have used enzyme linked immunosorbent assay (ELISA) to measure anti-filarial antibody against adult and microfilarial somatic antigens of *Brugia malayi* in patients with acute and chronic manifestations as well as occult form of the disease, tropical pulmonary eosinophilia (TPE).

Materials and Methods

Nine asymptomatic microfilaria carriers, 18 early filariasis patients, 15 chronic filariasis patients, 27 TPE patients, 15 normal healthy controls living in the same area (Madras) and 13 non-endemic controls from non-endemic area (Delhi) were studied.

Early filariasis patients had histories of episodes of acute lymphangitis and lymphadenitis, fever and pitting edema of the limbs (of duration not more than a year). The chronic filariasis patients had either elephantiasis or chyluria as a major manifestation of the disease. TPE patients presented with nocturnal cough, wheeze, breathlessness and high circulating eosinophil counts (> 2000/mm^3).

Brugia malayi adult worm (BmA) and microfilarial (Bm/mf/S) antigens were saline extracts of the sonicates of these worms. They were standardized for protein content as measured by Lowry's method[6].

ELISA was carried out according to Narayanan *et al* [7]. In brief, plates coated with antigen (2 µg/ml) in alkaline buffer were washed thrice and incubated with doubling dilutions (1/40 to 1/5120) of the sera for 30 min. The plates were washed again and incubated with 1/1000 dilution of antihuman IgG–horse radish peroxidase (HRP) conjugate (Cappel Labs., PA, USA). The buffer used for washing and dilutions was 0.02 *M* PBS (pH 7.2) with 0.05% tween-20 as a detergent. The plates were. washed after 30 min and further incubated with freshly prepared substrate orthophenylenediamine (0.002% w/v with 0.003% H_2O_2. The reaction was read at 30 min after addition of 8N H_2SO_4 to stop the reaction. The last dilution showing colour development was taken as the serum titre.

Results and Discussion

Anti-adult worm (BmA) and anti-microfilarial (Bm/mf/S) antibodies were detected in all filariasis patients, irrespective of the duration of the disease as well as parasitological status. The antibodies were also detected in endemic volunteers. The presence of antibodies in endemic volunteers does cast a doubt on the specificity of the antibody response. However, the nonendemic controls who have minimal chance of exposure to filarial parasites have very low antibody levels; thus validating the assay by ruling out non-specific binding of serum antibodies to the antigen. Since the binding is not of non-specific nature, the

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endemic volunteers either have anti-filarial antibody response of considerable magnitude or they have cross reacting antibodies. Similar findings have been reported using indirect fluorescence antibody test with *W. bancrofti* excretory-secretory antigen[8]. Earlier, Kaliraj *et al.* [9] also reported presence of antibodies in endemic normal controls. Since the antibodies are present in normal controls and almost absent in non-endemic controls, the antibody levels in the community could be considered as a parameter of endemicity of infection. Antibodies are present in serum always unlike microfilaria that are present only during night time, hence, making the night blood examination difficult. Hence, antibody measurement could be used as an alternative to night blood examination in epidemiological studies.

However, the relationship between antibody levels and rate of infection in community and the quantum of infection has not been studied as yet. Such studies should be done to evaluate the use of anti-filarial antibodies in seroepidemiology of filariasis.

Little difference was observed between antibody levels as measured by the adult or microfilarial antigen in any of the categories (Table 1). This may be explained by postulating that the antibody response is directed largely to the antigens that are common to two different stages of the parasite. It is difficult to explain the role of antibody responsein filariasis immunity as the response is neither directed to stage specific antigens nor related to the manifestations of the disease (either acute or chronic) except in case of TPE. Another aspect of antibody response in filariasis is the cross reactivity of filarial antigens with antigens of other helminthic parasites. We have demonstrated the cross reactivity of filarial antigens with ascaris antigens using TPE sera.

Table I – Anti-filarial Antibody Titres in Filarial Patients and Volunteers

Clinical category	No. of subjects	Mean IgG titres (range)	
		Anti-BmA	Anti-Bm/mf/S
Non-endemic normals	13	26 (0-1280)	6 (0-640)
Endemic normals	15	81 (0-1280)	62 (0-1280)
Asymptomatic mf carriers	9	62 (0-160)	86 (40-160)
Early filariasis	18	121 (40-1280)	208 (40-2560)
Chronic filariasis	15	221 (40-5120)	160 (40-1280)
Tropical pulmonary eosinophilia	27	2139 (80-5120)	2370 (80-5120)

Table 2 – Anti-filarial Antibody Titres in Filariasis Patients with or without Microfilaraemia

Microfila-raemia status	No. of specimens	Mean IgG titres (range)	
		Anti-BmA	Anti-Bm/mf/S
Mf +ve	22	65 (0-640)	119 (40-1280)
Mf –ve	18	235 (40-2560)	210 (40-2560)
P value		≤ 0.05	$\simeq 0.05$

In TPE individuals mean anti-filarial antibody titre is almost 10 times of that in controls and filariasis patients. This is in confirmity with findings of Neva and Ottesen[10]. Though mean antibody level in TPE is very high, a small proportion of TPE patients who otherwise confirmed to the clinical picture of TPE, had lower antibody titres. It is not known what this heterogeneity means in terms of aetiology and pathology of the disease.

Lastly, when the patients were classified on the basis of their parasitological status, those with circulating microfilariae had lower antibody titres compared with those without microfilaraemia (Table 2). Our findings are contrary to those of Grove and Davis[5] who could detect anti-microfilarial antibodies only in chronic filariasis patients (most of whom had become microfilaria negative). This could easily be explained as they had used whole microfilariae as the antigen and not sonicate of microfilariae as used by us. Hedge and Ridley [11] used the sonicate of *B. pahangi* and could detect antibodies in almost 100% of the patients studied. The lower levels of antibody titres in microfilaria positive patients may be due to absorption of antibodies onto the surface of microfilaria or may be due to the suppression of antibody production.

Though there was no association between the course of the disease (acute or chronic) in lymphatic form of flariasis, a high IgG response (in TPE sera) and relatively low antibody levels in microfilarimics were observed. The relevance of increased IgG levels in TPE needs further attention. Also, the significance and implications of low IgG antibody levels in a small proportion of TPE patients needs to be understood.

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