

Protective immunity in human Bancroftian filariasis: inverse relationship between antibodies to microfilarial sheath and circulating filarial antigens

B. RAVINDRAN, A.K. SATAPATHY, P.K. SAHOO & J.J. BABU GEDDAM

Division of Immunology, Regional Medical Research Centre, ICMR, Bhubaneswar, India

SUMMARY

The existence and the nature of protective immunity in human filariasis continues to be a subject of intense debate. While there is no broad consensus on functional immunity against larval and adult stage parasites, anti-microfilarial immunity has been demonstrated to be mediated by antibodies to the microfilarial sheath. In the present study, circulating filarial antigens (CFA), a marker of active filarial infection in human Bancroftian filariasis, was found to be inversely associated with antibodies to microfilarial sheath in a cohort of 411 subjects representing all categories of filariasis across the clinical spectrum of the disease. Approximately 80% of humans of all age groups (5–65 years) were found to have either CFA or anti-sheath antibodies. The inverse relationship observed between these two parameters was found to be independent of the clinical manifestation; both symptomatic and asymptomatic cases were found to display similar inverse association between CFA and anti-sheath antibodies. The prevalence of anti-sheath antibodies in the paediatric group was found to be very high as compared to adults; 78% of children below the age of 10 years tested positive for anti-sheath antibodies although the mf rate and CFA rate were only 4.5% and 22.7%, respectively, in this age group, indicating that developing larvae or juvenile adult stage parasites could have been the source of antigenic stimulus for induction of antibodies to the microfilarial sheath.

Keywords *W. bancrofti, filariasis, microfilarial sheath, anti-sheath antibodies, circulating filarial antigens*

Correspondence: B. Ravindran, Division of Immunology, Regional Medical Research Centre (ICMR), Chandrasekharpur, Bhubaneswar-751016, India (e-mail: immunol@dte.vsnl.net.in).

Received: 4 January 2000

Accepted for publication: 11 August 2000

INTRODUCTION

Lymphatic dwelling helminths, the causative agents of clinical filariasis in 120 million people, develop and survive in the lymphatics for several years. In the absence of empirical demonstration, the existence of protective immunity in human filariasis has been a subject of intense debate. While there is no broad consensus on acquired immunity against infective larval and adult stage parasites, anti-microfilarial immunity in human lymphatic filariasis is believed to operate through antibodies to the microfilarial sheath. The significant inverse association observed between the presence of antibodies to mf sheath and the absence of circulating mf both in Brugian (McGreevy *et al.* 1980) and Bancroftian filariasis (Ravindran *et al.* 1990) has been interpreted to indicate elimination of circulating mf by a process of antibody-dependent cell adherence (Subrahmanyam *et al.* 1978, Simonsen 1983). Similar associations observed in animal models earlier (Tanner & Weiss 1978) further strengthen the conclusion that antibodies to mf sheath could be instrumental in eliminating circulating mf.

During the course of our investigations on protective immunity in human filariasis, we observed that antibodies to mf sheath appear very early in the younger age groups well before the onset of microfilaraemia, an observation also recently reported from Tanzania (Simonsen & Meyrowitsch 1998) and this prompted us to examine the relationship between anti-sheath antibodies and the acquisition of filarial infection in endemic areas. We hypothesized that, as a consequence of appearance of anti-sheath antibodies, the host remains amicrofilaraemic rather than circulating mf being eliminated. We reasoned that studying the association between anti-sheath antibodies and circulating filarial antigen (CFA) in Bancroftian filariasis might be a useful indicator to address this issue. CFA is a definitive parameter of filarial infection in infected humans (Turner *et al.* 1993) and its prevalence in the population has been found to be about double the mf rate in several geographical areas (Weil

et al. 1996, Itoh *et al.* 1999). The findings of the present study indicate a very significant inverse association between the presence of CFA and the absence of antibodies to mf sheath and further suggest that anti-sheath antibodies could appear early during infection and presumably influence the maturation of adult filarial worms and the consequent patency.

MATERIALS AND METHODS

Study area and collection of blood samples

Clinical examinations and nocturnal blood surveys were conducted in four areas of Puri and Nayagarh district of Orissa State, India, which are highly endemic for Bancroftian filariasis (Ravindran *et al.* 1998, Sahoo *et al.* 2000). Parasitological examination of individuals was done by microscopic examination of Giemsa-stained finger prick blood smears (20 μ l) obtained by night blood survey. Each individual was examined clinically for acute or/and chronic disease manifestations of lymphatic filariasis. The following criteria were followed for classifying the study individuals into six categories: (i) elephantiasis/lymphoedema: patients presenting with persistent grade III nonpitting oedema, nonreversible on elevation with thickened skin (Surendran *et al.* 1996) for 5 years or more ($n = 35$); (ii) hydrocele: patients with persistent scrotal swelling of >6 cm for 5 years or more ($n = 44$); (iii) acute filariasis: patients in all age groups presenting with current or a history of one or more episodes of adenolymphangitis (ADL) with or without fever. Patients with chronic manifestations were excluded from this group ($n = 161$); (iv) microfilariae carriers: individuals with circulating microfilariae ($n = 44$); (v) cryptic infection: asymptomatic and amicrofilaraemic individuals with demonstrable antigenemia ($n = 30$), viz., circulating filarial antigen (CFA); and (vi) endemic normals: asymptomatic amicrofilaraemic individuals without antigenemia ($n = 97$). Approximately 5 ml blood was collected from the 411 individuals (age ranging from 5–65 years) belonging to the above six groups. The sera were separated and frozen at -20°C until use.

Immunoperoxidase assay

Antibodies to the microfilarial sheath of *W. bancrofti* were detected by indirect immunoperoxidase assay (IPA) as described by Ravindran *et al.* (1988). Briefly, acetone-fixed microfilariae purified from peripheral blood using 3 μ m polycarbonate membranes (Nucleopore Corporation, USA) were used for the assay. The antigen slides were pretreated for 20 min with 0.5% H_2O_2 in methanol to inactivate endogenous peroxidase activity. Approximately

15 μ l serum (1:5 diluted in PBS) was applied to the slides which were incubated in a humid chamber for two hours at 37°C . The slides were then washed three times with PBS and 15 μ l of goat polyvalent antihuman immunoglobulin–peroxidase conjugate (Cat. No. A-8400 Sigma Chemicals Co, St Louis, MO, USA) was added to the spots for detecting bound antibody activity. After washing the slides thrice in PBS, the reaction was visualized and scored using a light microscope after staining the slides with diaminobenzidine (50 mg/100 ml) in Tris-HCl buffer pH 8.6 with 1 μ l/ml H_2O_2 .

Detection of circulating filarial antigen (CFA)

The *W. bancrofti* antigen detection kit was used for detection and quantification of CFA. The tests were performed according to the manufacturer's recommendation (Tropical Biotechnology Pty Ltd, Townsville, Australia). Serum samples were boiled with EDTA, centrifuged and the supernatants were used for antigen testing. The results were expressed in arbitrary antigen units per milliliter using *O-gibsoni* antigen provided as standard in the kit (cut-off = 100 U/ml).

Statistical analysis

Geometric mean index (GMI) of mf density and CFA units were calculated using SPSS package for each group only for those cases which are positive for mf and/or CFA. Occasional samples with CFA units above 32000 U were taken as 32000 U since this was the detection limit of the kit.

RESULTS

The prevalence of CFA and/or anti-sheath antibodies in each of the six groups are shown in Table 1. Approximately 75–80% cases were positive for either CFA or anti-sheath antibodies in all the groups. Approximately 7.5% of the total cases were positive for both parameters; however, the frequency of double positives was significantly greater in 'cryptic infection' cases than in other groups. Very broadly, the subjects in filarial endemic areas could be classified into three groups based on anti-sheath antibodies: endemic normals and patients with elephantiasis were 'high responders', patients with ADL and hydrocele were 'moderate responders' and microfilarial carriers and subjects with cryptic filarial infection were 'low responders'. We had previously reported a similar distribution of anti-sheath antibodies in Bancroftian filariasis (Ravindran *et al.* 1990). However, at that time, a clear differentiation between 'endemic normals' and 'cryptic infection' could not be

Table 1 Prevalence of microfilaraemia, circulating filarial antigenemia and anti-sheath antibodies in relation to the filarial infection and disease spectrum

Category	No. in category	No. Mf* positive (%)	No. CFA** positive (%)	No. As-ab# positive (%)
Endemic normals	97	0 (0)	0 (0)	79 (81.44)
Elephantiasis/lymphoedema	35	0 (0)	3 (8.57)	28 (80.00)
Acute filariasis ADL	161	13 (8.07)	66 (40.99)	94 (58.38)
Hydrocele	44	3 (6.81)	15 (34.09)	24 (54.54)
Mf carriers	44	44 (100)	40 (90.90)	6 (13.63)
Cryptic infection	30	0 (0)	30 (100)	8 (26.66)

*Geometric mean Mf intensity per ml was 118.78, 547.72, and 382.28 in ADL, Hydrocele and Mf carriers, respectively. **Geometric mean CFA units was 226.86, 1734.52, 2337.91, 3710.70, and 1234.81 in Elephantiasis/Lymphoedema, Acute filariasis ADL, Hydrocele, Mf carriers, and Cryptic Infection, respectively. #AsAb, anti-sheath antibodies.

attempted because assay systems for detection of CFA were not available.

There was a very significant inverse relationship (chi-squared test, $P < 0.001$) between the presence of CFA and the absence of anti-sheath antibodies in both symptomatic (group 1) and asymptomatic (group 2) cases of filariasis (Table 2). Group 1 comprised symptomatic patients with acute filarial disease, ADL and those with chronic disease manifestations such as lymphodema/elephantiasis and/or hydrocele. Group 2 comprised an asymptomatic population, viz. (a) asymptomatic individuals with circulating mf, (b) asymptomatic, amicrofilaraemic individuals testing positive for CFA and (c) endemic normals, who are asymptomatic and amicrofilaraemic without CFA. The relationship was similar in both symptomatic and asymptomatic groups, indicating that the observed association between the two parameters is not dependent on manifestation of filarial disease. Approximately 80% of individuals in both the groups were found to have either CFA or anti-sheath antibodies. Approximately 7–8% were positive for both CFA and anti-sheath antibodies. This could have been due to polymorphic antigens expressed on the sheath of the heterologous mf used in the assay system and these sera are not likely to react with autologous mf (Ravindran *et al.*

Table 2 Correlation between anti-sheath antibodies and circulating filarial antigenemia among symptomatic and asymptomatic individuals in Bancroftian filariasis

	Symptomatic		Asymptomatic	
	No.	No. with antibodies (%)	No.	No. with antibodies (%)
CFA Present	87	16 (18.4)	67	14 (20.9)
CFA Absent	153	125 (81.7)	104	82 (78.8)

1994). Interestingly, the significantly high percentage of double positive cases amongst the ‘cryptic infection’ group in comparison to other categories suggests the higher prevalence of antibodies to polymorphic mf antigens in these amicrofilaraemic individuals. Approximately 11–12% of the sera tested in the study were negative for both parameters, which could be due to insufficient exposure to infection and noninduction of anti-sheath antibodies, or to technical limitations of either of the immunoassays.

Figure 1 shows the prevalence of CFA and anti-sheath antibodies in the same set of 411 human sera grouped and stratified according to age. Approximately 78% of children below the age of 10 years tested positive for anti-sheath antibodies. In this age group, the prevalence of CFA and microfilaraemia was only 22.7% and 4.5%, respectively. The high prevalence of anti-sheath antibodies seen in the younger age decreased with increasing age and CFA rate in the population. The prevalence of anti-sheath antibodies

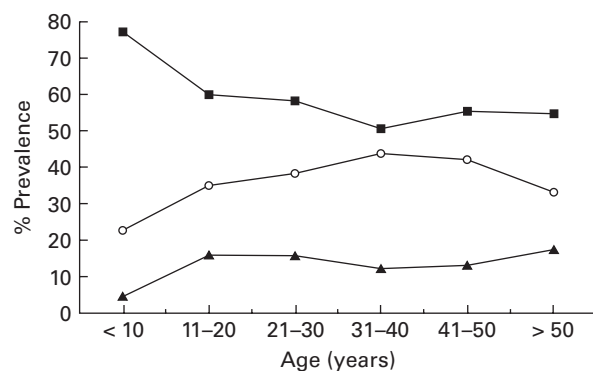


Figure 1 Prevalence of circulating filarial antigen (○), anti-sheath antibodies (■) and mf (▲) in 411 age stratified cases of Bancroftian filariasis. Each group consists of samples from both symptomatic and asymptomatic subjects. ($n = 22$ in < 10 years, $n = 100$ in 11–20 years, $n = 120$ in 21–30 years, $n = 73$ in 31–40 years, $n = 45$ in 41–50 years; $n = 51$ in > 50 years).

stabilized at approximately 55% after 30 years. The antigenemia rate, on the other hand, was 27.7% amongst children below 10 years, reaching a maximum of 42% in the age group 30–40 years and marginally declining in higher age groups. These findings indicate that sheath specific antibodies appear very early in children during exposure to filarial infection in human filariasis.

DISCUSSION

Simonsen & Meyrowitsch (1998) analysing a small number of samples in Tanzania found (i) an inverse association between IgG2 antibodies to the mf sheath and CFA, and (ii) a high prevalence (45.5%) of IgG2 anti-sheath antibodies in children below the age of 15 years in comparison to 16.7% positivity in adult population. While confirming the above findings, the current investigation also reveals the dynamics between CFA and anti-sheath antibodies in a large age stratified population. More crucially, this study demonstrates that the association between these two parameters is independent of clinical manifestations in human Bancroftian filariasis. The appearance of anti-sheath antibodies in 78% of the paediatric population (much before onset of antigenemia or microfilaraemia) suggests that the production of anti-sheath antibodies is not induced by microfilarial stages. The source of antigenic stimulus for induction of antibodies to sheath antigens before production of mf or maturation of adult worms in the host is currently not clear. It is possible, however, that some of the sheath antigens are expressed in developing larvae/juvenile adult stage parasites and induce anti-sheath antibodies (Maizels *et al.* 1983). The appearance of antibodies to mf sheath in early infection (during prepatent phase) has been observed in Mandrills infected with *Loa Loa* (Pinder *et al.* 1994). These authors suggested the possibility of induction of mf sheath-specific antibodies by the developing larvae/juvenile adult stage parasites, a possibility also suggested by Simonsen & Meyrowitsch (1998). This hypothesis is testable given the availability of cDNA expression libraries for L₃, L₄, adult male and female worms of *Brugia malayi*, increasing numbers of EST sequences and the gene sequences for some of the sheath antigens with the filariasis genome project (<http://helios.bto.ed.ac.uk/mbx/fgn/filgen1.html>).

The significant inverse association between CFA and anti-sheath antibodies and the observation that anti-sheath antibodies are produced very early during exposure suggests that approximately 80% of the population in filariasis endemic areas are committed to only one of these parameters, i.e. the individuals either display antibodies to mf sheath on being exposed to filarial infection or allow maturation of adult worms in their system resulting in CFA positivity. This association also explains the inverse relationship earlier

observed between presence of circulating mf and absence of antibodies to sheath in both Brugian and Bancroftian filariasis (McGreevy *et al.* 1980, Ravindran *et al.* 1990). Such associations were interpreted to mean that mf are eliminated from circulation on production of anti-sheath antibodies by the host. From the current study, it appears that the inverse association observed earlier between mf and anti-sheath antibodies was possibly due to nonmaturation of adult worms in anti-sheath positive individuals who were consequently amicrofilaraemic. However, it is pertinent to note that blood samples have been collected in the present study only on a cross-sectional basis. Long-term follow-up of the different groups for CFA and anti-sheath antibodies status over a period of time is expected to reveal the actual sequence of events in filarial infection/disease.

The inverse association observed between CFA and anti-sheath antibodies could mean that (i) such antibodies mediate elimination of adult worms (an argument applied earlier for anti-microfilarial immunity) or (ii) the antibodies to sheath antigens inhibit growth and development of larvae into mature adult worms. We prefer the second explanation since chronologically the antibodies appear early during infection in childhood as shown in this study and by that of Simonsen & Meyrowitsch (1998) and, currently, we have no mechanistic explanation as to how antibodies to sheath could mediate adult worm killing *in vivo*. These findings reported here thus suggest a protective role for anti-sheath antibodies in a broader perspective of anti-filarial immunity rather than anti-microfilarial immunity. Since the immunologically hyporesponsive state, characterized by down regulated Th1 responses in human filariasis, is associated with the presence of CFA (Dimock *et al.* 1996), it can be postulated that presence of anti-sheath antibodies (and absence of CFA) is indicative of a hyperimmune state in human filariasis. Current investigations in our laboratory are directed towards understanding the association between anti-sheath antibodies and production of IFN- γ by filarial specific T-lymphocytes in human Bancroftian filariasis.

ACKNOWLEDGEMENTS

The authors are grateful to Dr Nirupama Mishra, Research Officer, Central Homeopathic Research Unit, Puri; Mr Parikshit Pattanaik, President, GUC, Gania; Mr Pabitra Kumar Biswal of Siruli, Puri and Mr B.K.Pattanaik of Jatni PHC for helping at the time of sample collection in different villages. The Regional Medical Research Center, Bhubaneswar is funded by the Indian Council of Medical Research, New Delhi and the authors thank Dr K.Satyanarayana, Director of the Centre for his sustained support. This work was partly supported by a grant from European Commission (IC-18-CT 97–0245) to one of the authors (BR).

REFERENCES

- Dimock K.A., Eberhard M.L. & Lammie P.J. (1996). Th1-like anti-filarial immune responses predominate in antigen-negative persons. *Infection and Immunity* **64**, 2962–2967
- Itoh M., Weerasooriya M.V., Gunawardena N.K. *et al.* (1999) *Wuchereria bancrofti* antigenaemia in Sri Lanka. *Tropical Medicine International Health* **4**, 207–210
- Maizels R.M., Partono F., Sri Oemijati Denham D.A. & Ogilvie B.M. (1983) Cross-reactive surface antigens on three stages of *Brugia malayi*, *B. pahangi* and *B. timori*. *Parasitology* **87**, 249–252
- McGreevy P.B., Ratiwayanto S., Tuti S., McGreevy M.M. & Dennis D.T. (1980) *Brugia malayi*: relationship between anti-sheath antibodies and amicrofilaraemia in natives living in an endemic area of south Kalimantan, Borneo. *American Journal of Tropical Medicine and Hygiene* **29**, 508–513
- Pinder M., Everaere S. & Roelants G.E. (1994) *Loa loa*: immunological responses during experimental infections in mandrills (*Mandrillus sphinx*). *Experimental Parasitology* **79**, 126–136
- Ravindran B., Satapathy A.K. & Pattnaik N.M. (1988). Antibodies to diethylcarbamazine cross-react with microfilariae of *Wuchereria bancrofti*. *Immunology Letters* **17**, 7–11
- Ravindran B., Satapathy A.K., Das M.K., Pattnaik N.M. & Subramanyam V.R. (1990) Antibodies to microfilarial sheath in bancroftian filariasis – prevalence and characterization. *Annals of Tropical Medicine and Parasitology* **84**, 607–613
- Ravindran B., Satapathy A.K. & Sahoo P.K. (1994). Bancroftian filariasis - differential reactivity of anti-sheath antibodies in microfilariae carriers. *Parasite Immunology* **16**, 321–323
- Ravindran B., Sahoo P.K. & Dash A.P. (1998). Lymphatic filariasis and malaria: concomitant parasitism in Orissa, India. *Transaction of Royal Society of Tropical Medicine and Hygiene* **92**, 21–23
- Sahoo P.K., Babu Geddani J.J., Satapathy A.K., Mohanty M.C. & Ravindran B. (2000) Bancroftian filariasis: prevalence of antigenemia and endemic normals in Orissa, India. *Transaction of Royal Society of Tropical Medicine and Hygiene* **94**, 1–3
- Simonsen P.E. (1983). Immune reactions to *Wuchereria bancrofti* infections in Tanzania. I. Serum-mediated adherence of leucocytes to microfilariae *in vitro*, using serum from different groups of patients. *Transaction of Royal Society of Tropical Medicine and Hygiene* **79**, 853–858
- Simonsen P.E. & Meyrowitsch D.W. (1998) Bancroftian filariasis in Tanzania: specific antibody responses in relation to long-term observations on microfilaraemia. *American Journal of Tropical Medicine and Hygiene* **59**, 667–672
- Subrahmanyam D., Mehta K., Nelson D.S., Rao Y.V.B.G. & Rao C.K. (1978) Immune reactions in human filariasis. *Journal of Clinical Microbiology* **8**, 228–232
- Surendran K., Pani S.P., Soudarssanane M.B. *et al.* (1996) Natural history, trend of prevalence and spectrum of manifestations of bancroftian filarial disease in Pondicherry (South India). *Acta Tropica* **61**, 9–18
- Tanner M. & Weiss N. (1978). Studies on *Dipetalonema viteae* (Filarioidea). II. Antibody dependent adhesion of peritoneal exudate cells to microfilariae *in vitro*. *Acta Tropica* **35**, 151–160
- Turner P., Copeman B., Gerisi D. & Speare R. (1993). A comparison of the Og4C3 antigen capture ELISA, the Kontt test, an IgG4 assay and clinical signs, in the diagnosis of bancroftian filariasis. *Tropical Medical Parasitology* **44**, 45–48
- Weil G.J., Ramzy R.M.R., Ramaswamy C. *et al.* (1996). Parasite antigenemia without microfilaraemia in bancroftian filariasis. *American Journal of Tropical Medicine and Hygiene* **55**, 333–337