J. Biosci., Vol. 20, Number 2, March 1995, pp 167-174. © Printed in India.

Age-related prevalence of antibodies to infective larvae of *Wuchereria bancrofti* in normal individuals from a filaria-endemic region

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MS received 16 July 1994; revised 28 February 1995

Abstract. Antibody isotypic levels (IgM, IgE and IgG subclasses) to infective larvae (L_3) of *Wuchereria bancrofti* were measured in 104 normal individuals from a filaria-endemic region in Orissa. The titres of antibodies were considerably higher in adults (n = 25, 25·1 ± 3·8 year) than in children (n = 52, 7·1 ± 2·1 year). Young children (n = 14) less than four years were seronegative to all isotypes other than IgM, the sero-conversion to which was achieved in the children (n=15) by the age of 7·5±1·2 years. The prevalence of other isotypes increased with age and reached a maximum in early adulthood (18·6 ± 1·6 years), which persisted in older adults (> 30 years). However, the increase in IgG_3 prevalence with age was less marked. IgG_2 was detected only after 10 years of age. Compared to the high prevalence (100%) of IgM, IgE, IgG_1, and IgG_2, in adults. IgG_3 and IgG_4 prevalences were low, 35% and 28% respectively. IgA level to L_3 antigen was found to be extremely low even in adults. These data indicate that the prevalence of L_3 antibodies was different for different isotypes and the acquisition of antibody response essentially followed an age dependent pattern.

Keywords. Infective-larvae; *Wuchereria bancrofti;* antibody isotypes; age dependency; filariasis; endemic normals.

1. Introduction

People living in filarial regions can be classified into different distinct groups based on their clinical manifestations. These are asymptomatic microfilaraemia, chronic patients, with obstructive lesions such as elephantiasis and hydrocele and who may or may not be microfilaraemic; and endemic normals. Endemic normals are individuals apparently free from parasitaemia and clinical symptoms. The different clinical manifestations are thought to be due to differential immune responses of persons with filariasis. Infection is initiated by the infective larval (L_3) stage of the parasite during bites of the mosquito vector, *Culex quinquefasciatus.* Filariasis is a chronic disease, acquired after a prolonged exposure to infective bites in endemic regions.

The relationship of infection with the age of the host, the age-prevalence of microfilaraemia, has been described from the epidemiological data collected in India (Bundy *et al* 1991). The maximum rate of acquisition of filarial infection occurs in the 16-20 yr age group and then declines. Only recently have age-related immunological studies in filariasis been designed (Hitch *et al* 1989; Day *et al* 1991a). It has been shown that the development of antibodies to L_3 surface antigens

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which could be of importance in protective immunity requires prolonged exposure to infection (> 20 years) since the antibodies were detected mostly in adults (Day *et al* 1991a). The host protective immunity that might be acquired by adults against the L_3, is presumed to be effective against the acquisition of new infection. There has been practically no age-dependent immunological profiles in the normal population, a target group potentially important for evaluating protective immunity. The so-called "endemic normals" live in an endemic region but do not have parasitaemia or symptoms of the disease. The present study describes the age-dependent distribution of IgM. IgE and IgG subclasses to L_3, antigen in the normal population of an endemic region in Orissa.

2. Materials and methods

2.1 *Serum samples*

The study population was from a village (Bajapur) in Khurdha district, Orissa, India which is highly endemic (rate of microfilaraemia—15%) for *W. bancrofti* filariasis. The subjects have been studied earlier for the presence of Filarial (L_3) antigen using rabbit anti-L_3 serum by counterimmune electrophoresis (Das *et al* 1988a) and also by sandwich enzyme-linked immuno sorbent assay (ELISA) (M K Beuria and M K Das, unpublished observation), An antigen positivity varying from 8% (CIEP) to 20% (ELISA) was found in endemic normals.

Sera were prepared from 104 endemic normal individuals (L_3 antigen negative by ELISA) ranging in age from 1 to 50 years by finger prick blood collected in capillary lubes and stored at -20°C. The people were life-long residents in the area and were free from filarial symptoms. They had not been treated with diethyl carbamazine in the last seven years. The presence of microfilariae (MF) was determined by microscopic examination (Giemsa stained) of 20 µl blood taken between 8:30 pm and 11:30 pm. The individuals were checked twice in a span of one year for MF negativity. Control sera (n = 15) were also collected from adults (39·8 ± 8·5 yrs) living in non-filarial regions (Angul) of Orissa.

2.2 *Infective larval antigens*

Somatic antigen was prepared from L_3 larvae obtained from (adult female) *Culex quinquefasciatus* on day 14 of postmembrane feeding on microfilaraemic human blood. A soluble extract was prepared by homogenizing and sonicating (Branson Sonifer 450) the L_3 larvae for 5 min at 0°C in 0·01 M phosphate buffered saline (PBS), pH 7·2. The presence of *Culex* antigenic components was removed by adsorption with insolubilized rabbit antiserum against *C. quinquefasciatus* as described earlier (Das *et al* 1992). All L_3 antigenic extracts were prepared at the same time, divided into aliquots and frozen at -20^0C until used.

2.3 *ELISA*

Antibody levels to L_3 antigen were determined in sera following (he procedure of Das *et al* (1992). Mouse monoclonals anti-human IgM, IgG_1, IgG_2, lgG_3 and IgG_4; peroxidase conjugated goat anti-human IgA and peroxidase conjugated goat anti-mouse

immunoglobulins (IgS) were bought from Sigma Chemical Company, USA. Peroxidase conjugated rabbit anti-human IgG and IgE were from Dakopatts, Denmark. The wells of ELISA plate were coated with larval antigen (5 µg/ml, 100 µl) in 0·05 M carbonate buffer, pH 9·2, at 37°C for 4 h followed by incubating at 4°C overnight and then blocked with 0·5 % bovine serum albumin. Plates were washed with PBS-tween-20 (0·2%, 3x) followed by the addition of human sera (1/200 diluted). Each dilution was tested in duplicate. Plates were incubated for 3 h at 37°C and then washed again. Peroxidase -conjugated rabbit anti-human IgG and IgE (diluted 1/1000 in PBS-tween) was added, kept for 2 h at 37°C. After washing, OPD substrate (o-phenylenediamine containing H_2O_2) was added. The reaction was stopped after 30 min with 4 N H_2SO_4 and was read at 492 nm in a ELISA reader (Bio-Rad). Mouse monoclonal anti-human IgM, IgG_1, IgG_2, IgG_3 and IgG_4 (1/1000) were used followed by peroxidase conjugated goat anti-mouse IgS (1/1000) to quantitate the levels of the isotypes. The sera were checked from 1/25 dilution onward for determining the titre of IgE and IgG. The titre was designated as the reciprocal of the highest dilution of the serum which resulted in an absorbance value higher than the mean + 3 SD of a panel of non-tropical (North American) sera (n = 10, 1/25 dilution).

3. Results

L_3-specific IgM, IgG and IgE were initially determined at a fixed dilution (1/200) in children and adult endemic normals. IgG and IgE levels were found to be higher in adults than in children. The difference in IgM was not so marked although it was lower in children (data not shown). The IgG and IgE titres were determined (table 1). For the sake of comparison, the adult population was divided into two different age groups: I (20-30 years) and II (31-50 years). It is evident that adults have significantly (P <0·01) higher titre than children. There is no difference between the two groups of adults. The IgG response in general showed a high degree of inter-individual variation in both the groups.

The distribution of antibody titre in endemic normals is shown in figure 1. The number of individuals exhibiting a high titre antibody response was greater in adult

Table 1. Titre of *W. bancrofti* infective larval (L_3) antibodies in endemic normal population.

Group	n	Age (year) (Mean ± SD)	Titre[a] (range) IgE	IgG
Children	52	4–10 (7·1 ± 2·1)	100 ± 60 (50–200)	363 ± 312 (50–1600)
Adults (I)	25	20–30 (25·1 ± 3·8)	242 ± 121[b] (50–400)	772 ± 690[b] (50–3200)
Adults (II)	27	31–50 (42·8 ± 6·4)	252 ± 174[b] (50–800)	993 ± 900[b] (200–4800)

[a] Titre expressed as arithmetic mean ± 1 SD.

[b] Compared to children, P < 0·01.

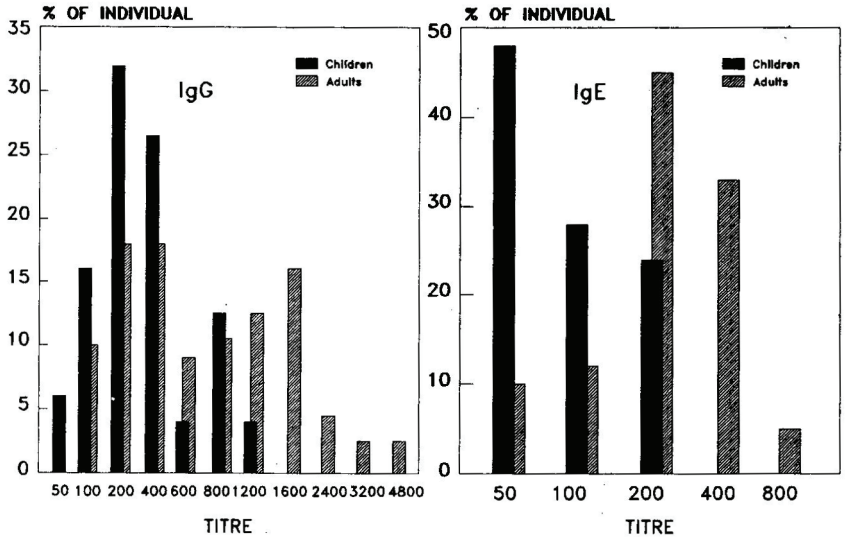

Figure 1. The titre of IgG and IgE antibodies to L_3 of W *bancrofti* in endemic normals (n=104)

groups. For example, 46% of adults compared to 16% of children have reciprocal IgG titres higher than 600. Similarly 76% of adults in contrast to 24% of children exhibited high (> 100) IgE titre.

The prevalence of antibody isotypes indicating the number of seropositive individuals in different age groups was determined (table 2 and figure 2). The rate of seropositivity for IgM peaked at a much earlier age (75 ± 1·2 yr). The seropositivity values for the other isotypes IgG_1, IgG_2, IgG_4 and IgE increased gradually with age and attained a maximum in early adult age of 16–21 (18·6± 1·6) years. These maximum values do not decline but persist into the older age groups (30+ years). However IgG_3 values did not differ significantly between children (15% positive) and early adult age (22% positive). IgG_3 prevalence attained a maximum of only 35% in adults. The prevalences of other isotype antibodies to L_3 larvae in adults (30 + years, n= 15) were as follows: almost all adults were sero positive to IgM, IgE, IgG_1, IgG_2 and 28% are positive to IgG_4. IgA levels in the sera, both of children and adults, were found to be extremely low (OD 492 < 0·02).

4. Discussion

The present study describes the dependence of antibody response to infective larvae of *W. bancrofti* with the age of individuals. The average infectivity rate of *C. quinquefasciatus* in this study area is 5·5% and an individual is likely to be infected with about 25,000 L_3 stage infective larvae in one year [Regional Medical Research Centre, Bhubaneswar, Interim Report (1994) on WHO/TDR Project "Field evaluation of *Bacillus sphericus* against *C. quinquefasciatus"].* People living in such a hyperendemic region will be continually exposed to the L_3 stage of filarial parasites and will elicit a specific immune response. This is supported by the observation that individuals from non-filarial yet tropical regions of Orissa elicited markedly

Table 2. Age-distribution of IgE and IgG subclass levels to infective larvae of *W. bancrofti*.

Age (years)	No. of sera	ELISA (OD 492)									
		IgE		IgG_1		IgG_2		IgG_3		IgG_4	
		Mean	Positive (%)	Mean	Positive (%)	Mean	Positive (%)	Mean	Positive (%)	Mean	Positive (%)
1–4 (3·3 ± 1·1)	14	0·01 ± 0·01	0	0·02 ± 0·02	0	0·02 ± 0·01	0	0·03 ± 0·01	0	0·01 ± 0·01	0
5–9 (7·5 ± 1·2)	15	0·03 ± 0·01	20	0·07 ± 0·03	33·3	0·06 ± 0·01	0	0·06 ± 0·01	13·3	0·036 ± 0·04	10
10–15 (13·1 ± 1·9)	13	0·05 ± 0·01	46	0·10 ± 0·02	53·8	0·07 ± 0·01	23	0·06 ± 0·03	15·3	0·05 ± 0·03	15·4
16–21 (18·6 ± 1·6)	14	0·07 ± 0·01	85·7	0·14 ± 0·02	78·5	0·10 ± 0·02	85·7	0·09 ± 0·04	21·4	0·09 ± 0·07	28·6

The sera were analysed at 1 : 200 dilution.
Cut off for IgE ≥ 0·05; IgG_1 ≥ 0·09; IgG_2 ≥ 0·08; IgG_3 ≥ 0·07; IgG_4 ≥ 0·08.

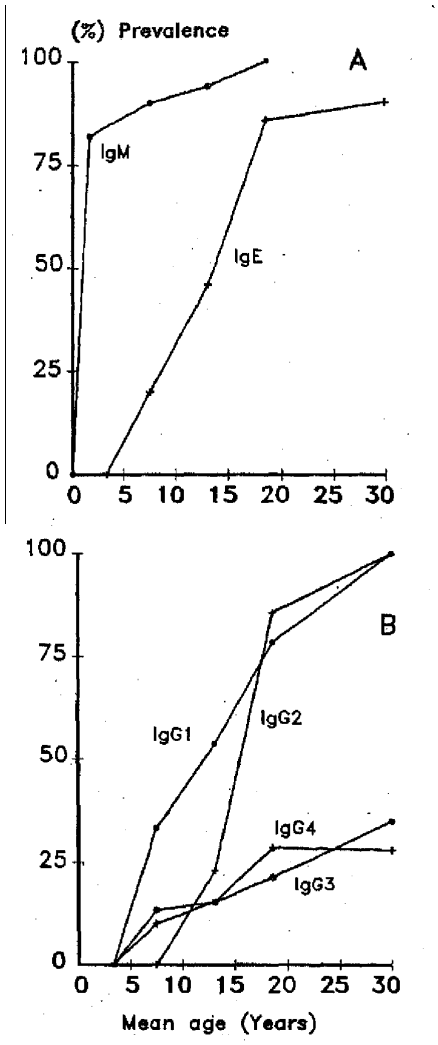

Figure 2. Age-dependency of prevalence levels for anti-L3 antibodies in individuals living in a filarial region. **(A),** IgM and IgE; **(B),** IgG subclasses. Isotypic positivity were calculated from the cut-of OD values. Cut of for IgM, OD≥ 0·10 and for other isotypes as mentioned in table 2. A sample is confirmed positive by two independent experiments.

reduced L_3 antibodies. These people may have been in all likelihood infected with parasites other than *W. Bancrofti.* The specificity of L_3 antibody response is thus emphasized.

The increase in the prevalence of anti L_3 antibodies of different isotypes as a function of the age of endemic normals indicates a chronic exposure driven immunity at antibody level. Full sero-conversion in IgM occurs around the 7th year but in other isotypes not until 18 years which indicates a constant exposure induced class switch. Prolonged exposure over several years seems to be a prerequisite to develop filaria- specific (IgE and IgG isotypic) immunity. Once attained, the L_3-isotypic response in endemic normals remains constant in older adults. Similar age dependency

of specific antibody isotypes has been described recently in human whipworm *(Trichuris trichiura)* infection (Needham *et al* 1992).

A comparison of L_3 antibody prevalence between endemic normals and the infected population asymptomatic MF-carrier and chronic patients (Das *et al* 1988b, 1992) does not suggest any clear-cut difference between the two. The levels of all antibody isotypes other than IgG_4 are elevated in chronic patients. Filaria-specific IgG_4, level is elevated only in MF-positive individuals (Kwan-Lim *et al* 1990; Das *et al* 1992) and it is particularly depressed in chronic patients (Hussain *et al* 1987). While both endemic normals and filaria-intected individuals have high prevalence (>90%) of IgE antibodies (Beuria and Das 1992), IgG_3 prevalence to L_3 is limited in endemic population. The prevalence in the endemic normals (35%) is similar to that in chronic (40%) or in asymptomatic (45%) filariasis, It has been reported that IgG_3, anlibody response is important in acquired immunity to Onchocerciasis (Boyer *et al* 1991). Many studies (Bloch and Malveaux 1985; Pancre *et al* 1988) implicated the effector role of IgE antibodies against parasitic nematodes *in vitro* and it is possible that they do have a protective taction in parasitic infection *in vivo* as well (Hagan *et al* 1991).

The results reported here find parallel in the antibody response to malarial parasites and schistosome (Nardin *et al* 1979; Hofman *et al* 1986; Butterworth and Hagan 1987; Woolhouse *et al* 1991), Although the mechanism of protective immune reactions in filariasis is not understood there are strong indications for the involvement of antibodies in mediating protective immunity (Kazura *et al* 1986; Freedman *et al* 1989: Praharaj and Das 1994), Age-related epidemiological studies have suggested that resistance to filarial reinfection may be acquired with increasing age (Bundy *et al* 1991: Day *et al* 1991b). It may be speculated that the age-related increase in larval antibody response as noted in endemic normals contributes towards the development of resistance against filarial infection.

Acknowledgement

We would like to thank Dr K Satyanarayana for interest.

References

Beuria M K and Das M K 1992 Immune response to an allergenic fraction *of Setaria digitata* in human filariasis; *J. Biosci.* **17** 453-461

Bloch E F and Malveaux F J 1985 The significance of immunoglobulin E in resistance to parasite infections; *Ann. Allergy* **54** 83-91

Boyer A E, Tsang V C W, Eberherd M L, Zea-Fores G, Hightower A. Pitcber J B, *Zea* Flores R. Zhou W and Reimer C B 1991 Evidence for IgG_3 involvement in acquired immunity to *Onchocerca volvulus* and identification of possible immune associated antigens; *J. Immunol.* **146** 4001-4010

Bundy D A P, Grenfell B T and Rajgopalan P K 1991 Immunoepidemiology of lymphatic filariasis: the relationship between infection and disease; *Immunoparasitol. Today* **10** 71-72

Butterworth E A and Hagan P I987 Immunity in human scistosomiasis; *Parasitol. Today* **3** 11-18

Das M K. Beuria M K, Dash A P and Pattnaik N M 1988a Detection of infective larval antigens in bancroftian filariasis: *Indian J Exp. Biol.* **26** 1003-1004

Das M K, Dash A P. Ravindran B. Pattnaik N M .and Subramanyam V R 1988b Quantitation of antibodies ío *infective larvae* in *Wurhereria bancrofti* filariasis; *Actaa Trop.* **45** 387-388

Das M K. Beuria M K and Dash A P *Immunoglobulin* E and G_4 antibodies infective larvae in a *Wuchererta bancrofti* endemic population. I*nt Arch. Allergy Immunol.* **99** 118-122

Day K P, Gregory W F and Maizels R M 1991a Age specific acquisition of immunity to infective larvae in a bancroftian filariasis endemic area of Papua, New Guinea; *Parasite Immunol.* **13** 277-290

Day K P, Grenfell B, Spark R, Kazura J W and Alpers M P 1991b Age specific patterns of change in dynamics of *Wuchereria bancrofti* infection in Papua, New Guinea; *Am. J. Trop. Med. Hyg.* **44** 518-527

Freedman D O, Nutman T B and Ottesen E A 1989 Protective immunity in bancroftian filariasis: Selective recognition of a 43 kD larval stage antigen by infection free individuals in an endemic area; *J. Clin. Invest.* **83** 14-22

Hagan P, Blumenthal U J, Dunn D, Simpson A J G and Wilkins H A 1991 Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium; Nature* (*London*) **349** 243-245

Hitch W L, Lammie P J and Eberhard M L 1989 Heightened antifilarial immune responsiveness in a Haitian Paediatric population; *Am. J. Trop. Med. Hyg.* **41** 657-663

Hoffman S L, Wistar R, Ballou W R, Hollingdale M R, Wirtz R A, Schneider I, Marwoto H A and Hockmeyer W T 1986 Immunity to malaria and naturally acquired antibodies to the circumsporozoite protein in *Plasmodium falciparum; N. Engl. J, Med.* **315** 601-606

Hussain R, Grogl M and Ottesen E 1987 IgG antibody subclasses in human filariasis. Differential subclass recognition of parasite antigens correlates with different clinical manifestation of infection; *J. Immunol.* **139** 2794-2798

Kazura J W, Cicirello H and Forsyth K 1986 Differential recognition of a protective filarial antigen by antibodies from humans with bancroftian filariasis; *J. Clin. Invest.* **77** 1985-1992

Kwam-lim G E, Forsyth K P and Maizels R M 1990 Filariae-specific IgG4 response correlates with active *Wuchereria bancrofti* infection; *J. Immunol.* **145** 4298-4305

Nardin E W, Nussenzweig R S, McGregor I A and Bryan J H 1979 Antibodies to sporozoites: their frequent occurrence in individuals living in an area of hyperendemic malaria; *Science* **206** 597-599

Needham C S, Bundy D A P , Lillywhite J E, Dider J M, Simmons I and Bianco A E 1992 The relationship between *Trichuris trichiura* transmission intensity and the age profiles of parasite specific antibody isotypes in two endemic communities; *Parasitology* **105** 273-283

Pancre V, Cesborn V Y, Auriault C, Joseph M, Chandenier J and Capron A 1988 IgE dependent killing of *Brugia malayi* microfilariae by human platelets and its modulation by T-Cell products; *Int. Allerg. Appl. Immunol.* **85** 483-486

Praharaj A K and Das M K 1994 Enhanced antibody response to a detergent-soluble antigen in human filariasis after treatment with diethyl carbamazine; *J. Biosci.* **19** 201-206

Woolhouse M E J, Taylor P, Matanhire D and Chandiwana S K 1991 Acquired immunity and epidemiology of *Schistosoma haematobium; Nature* (*London*) **351** 757-758