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Age-specific acquisition of immunity to infective larvae in a bancroftian filariasis endemic area of Papua New Guinea

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Summary The development of antibodies to infective stages of the filarial parasite, *Wuchereria bancrofti*, with age of the host human population was studied by immunofluorescence, immunoprecipitation and immunoblotting assays. Among individuals under 20 years of age, few had detectable antibodies to the infective (L3) larval surface by IFA: only 2 out of 10 scored positive. However, all adults (over 20 years) were positive in this assay although the utilization of isotypes varied between different individuals. Whilst antibodies to the L3 surface are therefore acquired after prolonged exposure to infection (> 20 years), recognition patterns of L3 surface labelled antigens, measured by immunoprecipitation analysis iodinated proteins on SDS-PAGE, and of somatic L3 proteins on immunoblots, were equivalent in the two age groups. Thus, a critical surface antigen, recognised in an age-dependent manner, is present on the L3 cuticle but cannot be resolved as a conventional protein or glycoprotein constituent.

Keywords: filariasis, human, immunity, age, third-stage larvae, fluorescence, Ig isotypes, Western blot, immunoprecipitation, surface antigens, surface labelling

Introduction

Residents of bancroftian filariasis endemic areas are continually exposed to infection with mosquito transmitted infective larvae (L3), some of which survive to become adult worms in the human host. It is unclear whether such life-long exposure to L3 induces immune-mediated resistance to reinfection with this life-cycle stage. Very few epidemiological studies have been designed to address the issue of resistance to filarial infection in humans. Estimation of age-specific rates of gain and loss of *Wuchereria bancrofti* infection from epidemiological data collected in Pondicherry, India showed that the rate of gain of infection exhibits a convex age profile peaking in the 16–20 year age class (Vanamail *et al.* 1989). The reduced rate of gain in adults above 20 years may be accounted for by acquisition of resistance to new infection.

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Another epidemiological study of a bancroftian filariasis endemic area of Papua New Guinea described the age-specific dynamics of *W. bancrofti* infection in this community (Day *et al.* 1990). Striking differences in the dynamics of adult worm populations, as assessed by circulating phosphorylcholine (PC) containing antigen levels, were observed for subjects $\leqslant 20$ years in comparison to those > 20 years. Adult worm burdens were observed to increase in the younger age group in comparison to the older age group during the 12 months of the study. These data were considered in the context of a mathematical model describing the dynamics of adult worm population in relation to their life expectancy and attrition of larvae during establishment. This analysis showed that the observed patterns of change in intensity of infection with age were consistent with the acquisition of resistance to new infections with increasing experience of infection. Furthermore, this analysis indicated that this resistance was likely to be directed at the early larval stages of *W. bancrofti* rather than adult worms. This interpretation of the epidemiological data fits well with evidence from trickle infection experiments in *Brugia pahangi* infected cats (Denham *et al.* 1983), an important animal model for human filariasis. These experiments have shown that adult worms in persistent infections are relatively long-lived (with a life expectancy of around two years) and arise mainly from early infection when the hosts are essentially naive. Later in the course of infection, these hosts are able to destroy 90% or more of incoming larvae within 24 hours of injection.

If repeated exposure to L3 induces such resistance in areas of stable *W. bancrofti* transmission, and this resistance is immune-mediated, age dependent acquisition of immune responses to L3 should be observed. A number of studies have reported the presence of antibodies to the L3 surface of lymphatic filariae in humans (Wong & Guest 1969, Higashi & Chowdhury 1970, Subrahmanyam *et al.* 1978, Sim, Kwa & Mak 1982). The influence of host age on the response to these L3 surface antigens has never been recorded. Indeed, to date, there have been very few seroepidemiological investigations characterizing the age-specific immune response to any filarial antigens (Weiss & Karam 1989). To test this hypothesis, sera from subjects aged 6 to 55 years of known parasitological status were screened for antibody reactivity to the surface of living *B. malayi* L3. The closely-related filarial species *B. malayi* was chosen for these experiments due to lack of availability of *W. bancrofti* L3. The limited amount of information so far obtained on the surface antigens of both *W. bancrofti* (Maizels *et al.* 1986) and *Brugia* species (Maizels *et al.* 1983, Carlow *et al.* 1987, Lal & Ottesen 1988) infective larvae indicates a considerable antigenic homology, and the results presented below implicate an important surface specificity, shared between these species, as a possible target of protective immune responses in humans.

Materials and methods

STUDY POPULATION

Life-long residents of the Dreikikir area of East Sepik Province, Papua New Guinea, participated in a longitudinal study of the dynamics of *W. bancrofti* infection in June 1984. Details of this study as well as a description of this bancroftian filariasis endemic area are given elsewhere (Day *et al.* 1990). Parasitological status of the study population was determined by Nucleopore filtration of night blood samples for detection of

microfilariae as described previously (Day *et al.* 1990). Blood for serum was also collected during night blood surveys. Sera were stored at −40°C until analysed.

SEROLOGY

Serum samples were screened for PC-containing antigens by an immunoradiometric assay using the PC-specific monoclonal antibody designated Gib13 as described previously (Forsyth *et al.* 1985). Results of assays were expressed as an antigen index (AI).

$$\text{AI} = \frac{\text{counts per min bound with test serum}}{\text{counts per min bound with control serum pool}}$$

The control serum pool was made up of 10 sera from residents of Melbourne, Australia who had never visited a filariasis endemic area. Sera from all Mf-negative subjects were also screened for a 200000 kDa PC-containing antigen by immunoadsorption and Western blotting as described previously (Day *et al.* 1990). Presence of the 200000 kDa PC-containing antigen in the sera of these amicrofilaremic subjects verified the *W. bancrofti* origin of PC-containing material detected by Gib13 IRMA. Serum samples were screened for antibodies to a somatic extract of *B. malayi* by ELISA as described previously (Day *et al.* 1990).

IMMUNOFLUORESCENCE

Viable *B. malayi* infected larvae (L3) were isolated from *Aedes aegypti* 12 to 14 days after being membrane fed on blood mixed with microfilariae taken from intraperitoneally infected jirds (*Meriones unguiculatus*) (McCall *et al.* 1973). Larvae were washed three times in RPMI 1640 (Gibco) and 50 living L3 in 95 μl of this medium were aliquoted into 0·5 ml plastic centrifuge tubes. Five μl of each test serum was added to a tube containing L3. Sera were tested in duplicate. Tubes were incubated for 1 h on ice. Unbound antibody was removed by washing L3 with human tonicity phosphate buffered saline (HTPBS) by gentle centrifugation at 100 *g* for 2 min. In initial experiments, FITC-sheep anti-human Ig (Sigma) diluted 1 : 50 in HTPBS containing 0·01% Evans' Blue and 10% foetal calf serum (FCS) was added to washed L3 and incubated for 1 h on ice. L3 were then washed 3 times and examined by UV illumination. To determine the class or subclass of antibody binding to the L3 surface, immunoglobulin heavy chain specific mouse monoclonal antibodies (Unipath) diluted 1 in 50 in HTPBS containing 10% FCS were added to washed L3 and incubated for 1 h on ice. Unbound monoclonal antibodies were removed by washing 3 times in HTPBS as above. L3 were then incubated for 1 h on ice with 100 μl of a 1 in 50 dilution of a fluorescein-conjugated anti-mouse immunoglobulin reagent (Sigma) in HTPBS containing 0·01% Evan's blue and 10% FCS. L3 were then washed 3 times in HTPBS as above and examined by UV illumination. At least 20 L3 were examined for each serum incubation. A drop of Citifluor (City University) was added to L3 prior to examination for fluorescence.

L3 ANTIGENS

For 1-dimensional immunoblot analysis, 2000 *B. malayi* L3 were taken up in SDS-PAGE loading buffer (Studier 1973) containing fresh 5% 2-ME (Sigma) and incubated at 100°C

for 5 min. The suspension was then homogenized and again incubated for 5 min at 100°C, before centrifugation. The supernatant was loaded onto a 5–25% SDS-PAGE gradient gel, electrophoresed and electroblotted onto nitrocellulose paper on an LKB Novablot apparatus. Following transfer, filters were blocked overnight in 5% low fat milk, and then cut into 4 mm strips and probed with human serum samples diluted 1 in 100 in PBS, 5% low fat milk, 0·05% Tween-20 for 2 h. After washing, strips were exposed to a 1 in 100 dilution of a polyvalent rabbit anti-human Ig serum for 1 h, washed again, and incubated for 2 h with 10^6 cpm/ml of ^{125}I-labeled Protein A (Sigma). Blots were washed extensively for 8 h before exposure for autoradiography on X-ray film.

SURFACE IODINATION

Five hundred *B. malayi* were subjected to surface directed radio-iodination with Bolton-Hunter reagent as described previously (Maizels *et al.* 1983, 1986) and solubilized in 1·5% n-octyl glucoside (Sigma) in PBS containing a cocktail of protease inhibitors. The supernatant recovered from a 10000 ***g*** centrifugation, was analysed directly by gradient SDS-PAGE, or taken for immunoprecipitation analysis with human serum antibodies. For this, 2×10^5 cpm of labelled protein was precleared by incubation with 5 μl of Protein A-Sepharose. The nonadherent material was mixed with 2·5 μl human serum in 50 μl, PBS, 0·5% Triton X-100, and incubated at 4°C overnight. Then, 2·5 μl of rabbit anti-human Ig was added, followed 30 min later by 20 μl of Protein A-Sepharose. Immune complexes were precipitated by centrifugation, washed three times, and dissociated in Studier buffer (Studier 1973) for analysis by gradient SDS-PAGE.

Results

DEMOGRAPHIC, PARASITOLOGIC AND IMMUNOLOGIC FEATURES OF THE STUDY POPULATION

Sera of the three groups of life-long residents of a bancroftian filariasis endemic area of Papua New Guinea were screened by IFA to examine the antibody response to the surface of L3. Demographic (age/sex), parasitological (Mf density and Antigen Index (AI)) and immunological characteristics of these groups are shown in Table 1. Individuals in Group A were aged between 6 and 15 years of age. Only four out of ten were microfilaraemic. The Mf densities of these subjects ranged from 3 to 1833 Mf/ml blood. All Group A subjects had detectable PC-containing antigens by Gib 13 IRMA, with AI $\geqslant 2{\cdot}0$. The mean AI of the group was $3{\cdot}4 \pm 0{\cdot}5$. Group B subjects were aged between 22 and 50 years of age. Seven of these ten adults were microfilaraemic with Mf densities ranging from 1 to 3 Mf/ml blood. Again all Group B subjects had detectable PC-containing antigen by Gib13 IRMA. The mean AI of Group B was $4{\cdot}5 \pm 0{\cdot}9$. Group C subjects were aged between 25 and 55 years of age and were all microfilaraemic with Mf densities ranging from 192 to 9225 Mf/ml blood (geometric mean Mf density = 1845 Mf/ml blood). Group C subjects were all antigen-positive by Gib13 IRMA with a mean AI for the group of $10{\cdot}5 \pm 1{\cdot}1$. The amicrofilaraemic subjects listed in Table 1 can be assumed to have active *W. bancrofti* infection as detection of PC-containing antigen in circulation is a reliable indicator of filarial infection in this endemic area (Forsyth *et al.* 1985). As both Mf density and levels

Table 1. Demographic and parasitological features of the study population

Code No.	Age/sex	Mf/ml blood	Antigen index	ELISA
A				
81	15 M	603	2·8	0·48
82	9 F	1833	7·6	0·32
83	12 M	1722	4·4	0·41
84	10 F	0	2·3	0·75
85	9 M	0	2·8	0·67
86	15 M	0	3·4	0·44
87	15 F	3	2·5	0·21
88	12 M	0	3·1	0·72
89	6 F	0	2·4	0·65
90	6 F	0	2·9	0·50
B				
1	27 F	1	5·7	0·73
2	25 F	3	1·1	0·61
3	50 M	0	2·6	1·60
4	42 F	1	8·1	1·23
5	39 F	1	4·6	1·19
6	39 F	1	4·6	1·29
7	22 F	0	2·8	0·63
8	36 F	0	2·5	1·62
9	29 F	2	10·0	1·31
10	48 M	3	2·6	0·41
C				
19	27 F	2337	6·2	0·41
20	35 F	192	13·9	1·63
21	25 M	250	15·4	1·19
22	25 F	1624	12·3	1·54
23	36 F	3813	13·6	1·12
24	36 M	3198	9·2	0·88
25	41 F	2829	10·7	0·63
26	46 M	2214	5·3	1·00
27	39 F	3567	12·3	0·33
28	55 M	9225	6·5	0·37

of PC-containing antigen are indirect measures of *W. bancrofti* adult worm burden (Day *et al.* 1990), Group C individuals clearly have higher worm burdens than do Group A and B subjects.

Further evidence of filarial infection in this study population can be inferred from the ELISA data reported in Table 1. All subjects had detectable levels of IgG antibodies to an extract of *B. malayi* adult worms.

ANTIBODY TO L3 SURFACE IN IMMUNOFLUORESCENCE ASSAYS

Figure 1 summarizes immunofluorescence assay (IFA) data for sera screened at one in twenty dilution against the surface of living *B. malayi* L3. Despite evidence that all subjects in Group A were infected with *W. bancrofti*, only two of the ten sera were scored IFA positive. The two antibody-positive subjects were amicrofilaraemic and were not the eldest of their group. In contrast, all adult sera (Groups B and C) scored positive at the same serum dilution. Fourteen out of 20 of these IFA reactions with adult sera were scored 3+. There was no relationship between IFA reactivity and apparent worm burden in adults as the prevalence of positive reactions was 100% in both group B and C. The class/subclasses of antibodies recognizing the surface of *B. malayi* L3 were analysed using heavy chain-specific anti-immunoglobulin reagents (Table 2). Only eight (including children's sera 84 and 89) of the 22 sera scored as positive in Figure 1 were reanalysed in IFA reactions with these reagents. The predominant class of antibody to the L3 surface in all eight positive sera was IgM. Whilst all IFA positive sera contained specific IgM the pattern of IgG subclasses of antibody recognizing the L3 surface was subject to individual variation. For example serum 24 contained specific antibodies of all IgG subclasses whereas serum 1 contained only antibodies of the IgG_1 subclass. Seven of the eight sera contained specific antibodies of one or more of the IgG subclasses.

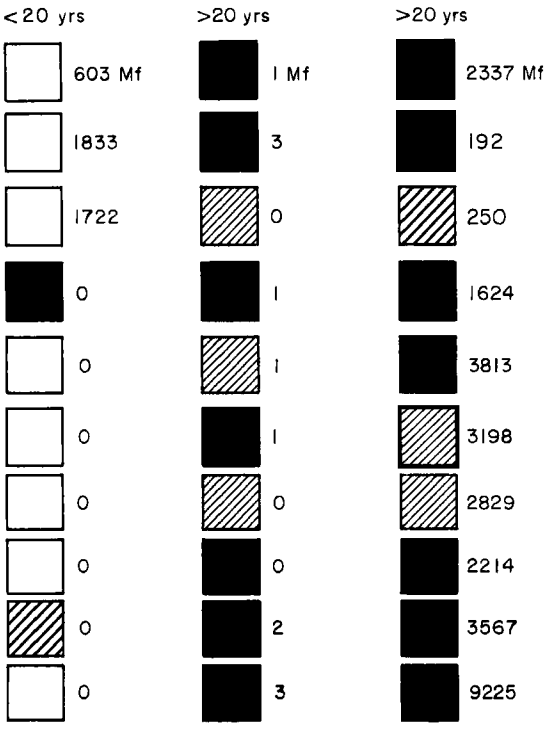

Figure 1. IFA reactivity of 30 human sera from residents of a *W. bancrofti* endemic area of Papua New Guinea. Sera were tested at a 1 in 20 dilution for binding to the surface of living *B. malayi* L3. □: Negative; ▨: 1+; ▩: 2+; ■: 3+.

RECOGNITION OF L3 ANTIGENS

In an attempt to identify the individual surface antigen(s) involved in the age-dependent differential recognition demonstrated by IFA, a series of experiments were carried out with somatic and surface proteins derived from *B. malayi* L3. This heterologous species was shown in the IFA study described above to share the surface antigen specificity with *W. bancrofti.*

The identification of this target antigen was first attempted with surface-iodinated proteins from live L3, mediated by the Bolton-Hunter reagent (BHR) which had previously been shown to label a wide range of surface associated molecules (Maizels *et al.* 1983, 1986). A detergent-soluble extract of BHR-labelled *B. malayi* L3 was taken for immunoprecipitation analysis, in a system which combined polyvalent anti-human Ig with protein A to precipitate IgM as well as IgG. A range of labelled proteins were precipitated by infected human sera, and in particular antigens migrating at 15, 24, 35, 44 and 49 kDa were recognized by nearly all individuals in the study (Figure 2). However, the immunoprecipitation profiles showed no quantitative or qualitative difference between sera containing antibodies reactive to the L3 surface in IFA (Panel A) and those devoid of anti-surface reactivity (Panel B). The same conclusion emerged from comparisons of individual sera or of serum pools (Figure 2). Two-dimensional analysis was also undertaken, but no difference was observed between the two serum pools (data not shown). In particular, neither group precipitated a 17–200 kDa complex of neutral pI associated with the surface of mammalian stages of *B. malayi* (Maizels *et al.* 1989). Similar findings were obtained when the more restricted range of surface proteins labelled by the Iodogen technique were evaluated (data not shown).

A further approach taken was to study reactivity of the IFA positive and negative sera in immunoblot analysis with whole somatic extracts of infective larvae, prepared by SDS-2ME solubilization. This may help identify targets which are not normally labelled by extrinsic techniques or those which are not isolated by mild detergent extraction. Individual serum samples were tested on immunoblot strips, which were probed to reveal

Table 2. Isotype distribution of L3 surface-reactive antibody

Code no.	IgG_1	IgG_2	IgG_3	IgG_4	IgM
84	–	1+	–	–	2+
89	–	–	–	–	1+
1	3+	–	–	–	3+
9	3+	–	–	–	3+
10	2+	–	2+	–	3+
19	1+	2+	–	–	3+
23	1+	3+	–	1+	3+
24	3+	2+	3+	2+	3+
Melbourne control	–	–	–	–	–

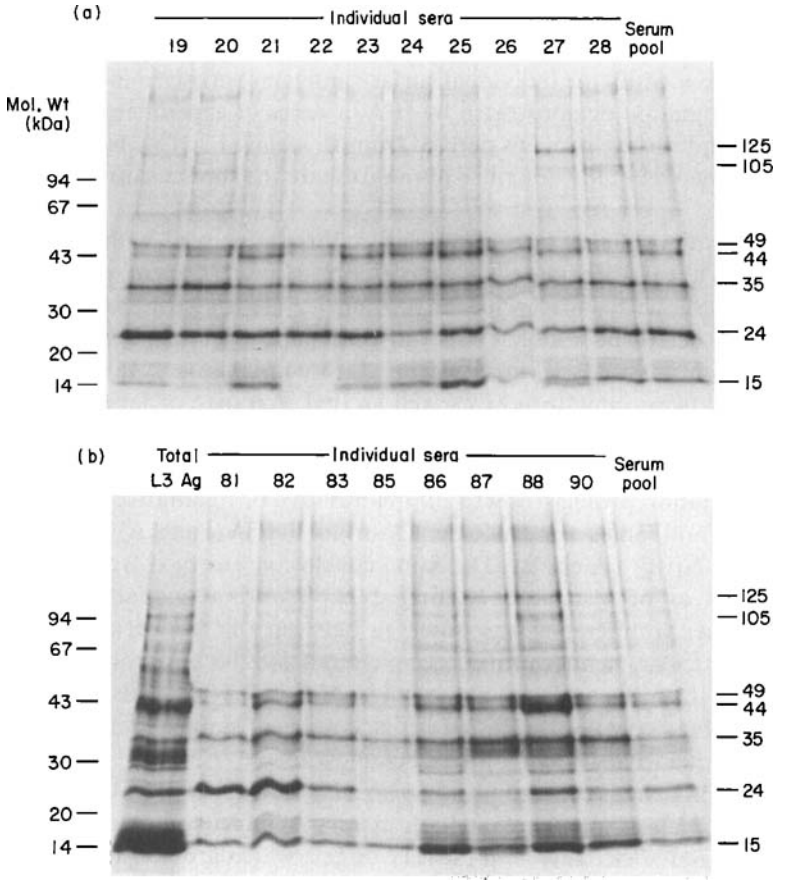

Figure 2. Immunoprecipitation analysis of BHR-labelled surface antigens from *Brugia malayi* infective L3 larvae. Panel a, individual adult sera code numbers 19 to 28 (see Table 1). The extreme right hand track shows the reaction of a pool of all 10 sera. Panel b, individual children's sera, code numbers 81 to 90, excepting 84 and 89 which were IFA positive. The extreme right hand track shows the reaction of a pool of all 8 IFA-negative sera. Total BHR-labelled L3 antigens are shown in the extreme left-hand track. The positions of molecular weight markers are indicated on the left hand margins; the molecular weights of major *Brugia malayi* L3 products are indicated on the right hand margins.

all isotypes of human antibody, and the results are presented in Figure 3. Despite extensive recognition of parasite antigens by the adult infected sera, no consistent differential pattern could be discerned between the antibodies with and without surface binding activity. Nearly all subjects showed reactivity to major immunogens of 15, 33, 62–70, 94, 105 and 200–210 kDa. However, few individuals reacted to 17–18 and 22 kDa bands, which have been reported elsewhere to evoke antibodies only in *Brugia*-infected cats that had developed immunity to repeated infection (Fletcher *et al.* 1986). Weak and inconsistent binding was seen towards a 43 kDa antigen, which has been reported (Freedman, Nutman & Ottesen 1989) to be strongly recognized by uninfected, putatively

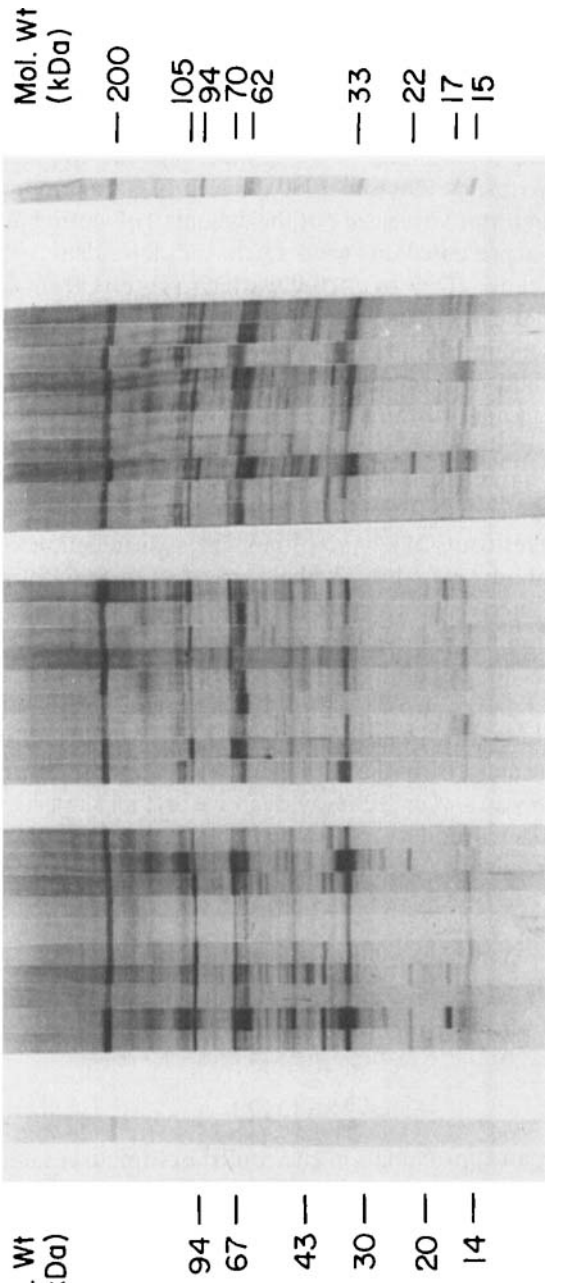

Figure 3. Western blot analysis of total soluble worm extract from L3 larvae of *Brugia malayi*. Normal sera and *B. malayi*-infected serum controls are shown at either end. The results with individual *W. bancrofti*-infected human sera are presented for adult, low microfilaraemics (code numbers 1–10), adult high microfilaraemics (codes 19–27), and children (codes 81–90). The strips corresponding to the two IFA-positive sera, 84 and 89, are indicated with arrows. The positions of molecular weight markers are indicated on the left hand margin; the molecular weights of major *Brugia malayi* L3 products are indicated on the right hand margin.

immune, endemic normal cases; no individuals in this category were present in the current study.

Discussion

The natural level of control of filarial infection in residents of endemic areas has long been an enigmatic question, with no clear evidence of naturally immune individuals, and a dissociation between severe pathological symptoms such as elephantiasis, and the presence of circulating parasites (Ottesen 1989). Recently, using a circulating antigen assay as a noninvasive quantitative measure of the dynamics of worm burdens in infected humans, evidence has been presented that suggests that adult residents of an endemic area possess a degree of resistance (Day *et al.* 1990) which protects them from continuing infection with L3 while not eliminating mature worms parasitizing the same individuals. Thus a situation of concomitant immunity may exist as has been described for other helminth parasites (Smithers & Terry 1976). These new data implied that an age-related immunological distinction may be found in reactivity towards the infective larvae. We now present an experimental demonstration that such a key age-related immunological difference exists, although there is as yet no direct evidence that recognition of the larval surface antigen described here is protective to humans.

IFA data showed that residents of a bancroftian filariasis endemic area of Papua New Guinea acquire an antibody response to the surface of mosquito-derived L3 with increasing experience of infection as reflected in their age. Prolonged exposure to L3 was necessary to induce this immune response since adults ($\geqslant 20$ years) but not juveniles (aged 6 to 15 years) were found to be antibody-positive by IFA. From entomological data collected in the endemic area (Bryan 1986) it is possible to make an estimate of the number of L3 (N) required to induce anti-L3 surface antibodies. To calculate N it is necessary to define the age at which the majority of the population become antibody positive. Since the majority of subjects < 15 years were antibody-negative and all subjects > 22 years were IFA antibody-positive it is reasonable to assume that a mimimum of 20 years of intense exposure to infection is required to develop antibodies to the L3 surface in the majority of the population. Thus N can be calculated as follows:

$$N = L \times F \times M \times 365 \text{ days} \times 20 \text{ years}$$

where L = mean number of L3 per infective bite, F = the frequency of infective bites and M = the number of man bites per night. From previously published data (Bryan 1986) $L = 2$, $F = 0{\cdot}035$ and $M = 20$.

$$\text{Thus } N = 10\,220.$$

This figure is obviously an approximation and could be estimated more accurately if the age-specific immune response to the L3 surface were analysed with a larger number of samples than we have used in these experiments.

The IFA response to the L3 surface was not induced by infection with mature worms and/or microfilariae since children who had evidence of active *W. bancrofti* infection were antibody-negative. The inability of these life-cycle stages to induce a cross-reactive immune response to the L3 surface in humans indicates that stage-specific epitopes are expressed on the L3 surface. The intense exposure to L3 required to induce this antibody response also implies that these epitopes are both stage-specific and lost very shortly after

infection. The existence of stage-specific antigens/epitopes on the L3 surface was first indicated by immunofluorescent antibody assays (Wong & Guest 1969), and has now been confirmed by surface-labelling studies (Maizels *et al.* 1983, Lal & Ottesen 1988), immunization experiments with L3 (Weiss & Tanner 1981) and with monoclonal antibodies (Carlow *et al.* 1987). Whilst the possibility also exists that the exposure-dependent antibody response to the L3 surface is stimulated to mosquito-derived material coating the L3s, experiments in animal models of lymphatic filariasis support the association between anti-surface antibody and protection against infection. Furthermore any vector-associated antigen would have to be shared between the anopheline species responsible for transmission, and the laboratory maintained *Aedes aegypti*.

Immunization with radiation-attenuated *B. malayi* and *B. pahangi* larvae in BALB/c mice (Hayashi *et al* 1984, Abraham *et al.* 1989), jirds (Chusattayanond & Denham 1986, Yates & Higashi 1985, 1986) and cats (Oothuman *et al.* 1979) protects animals against subsequent challenge with living L3. In the rodent models at least, protection was associated with an antibody response to the L3 surface (Yates & Higashi 1985, Abraham *et al.* 1989). It is interesting to speculate that the process of attentuation allows longer exposure to the host immune system of L3 surface antigens which are not ordinarily immunogenic. Trickle infection experiments in *B. pahangi* infected cats, a natural host of this filarial parasite, demonstrate that cats acquire resistance to L3 with prolonged exposure to this life cycle stage (Denham *et al.* 1983). This acquired resistance is initially stage-specific since adult worms are relatively long-lived in trickle-infected cats but most newly injected L3 are killed only 24 hours after injection. The role of antibodies to the surface of L3 in mediating this killing unknown and awaits further exploration. It has been reported, however, that the passive transfer of immune sera will protect jirds on subsequent challenge (Higashi & Yates 1987).

Individual variation in the class and subclass of antibody induced in adults was observed. Although all adults had a detectable IgM antibody response to the surface of L3 only some produced antibody responses of the IgG class. It is presumed that either class of antibodies may play a role in protection against invading L3 by antibody-dependent-cell-mediated-cytotoxicity reactions. Such reactions against L3 have been demonstrated *in vitro* (Higashi & Chowdhury 1970, Sim *et al.* 1982), but not as yet *in vivo* (Tanner & Weiss 1981a, b).

It is noteworthy that the L3 surface antigen identified by adult human filariasis sera is cross-reactive between the filarial species *Wuchereria bancrofti* and *Brugia malayi*. Similar cross-reactivities have been shown between surface of adult filarial worms (Maizels *et al.* 1985). The possibility therefore exists that a protective immunogen may be found which is effective against a range of parasites in this group. In this context it is important to recall the findings that irradiated *B. pahangi* L3 protect cats equally well against *B. pahangi* or *B. patei* (Oothuman *et al.* 1979), and that immunization of jirds with irridated L3 of a rodent filaria, *Litomosoides carinii*, gave extremely potent protection against challenge with *B. malayi* L3 (Storey & Al-Mukhtar 1982).

The implication of our results here is that the appropriate antibody response to the L3 surface may protect humans against filarial infection. Thus, it would be of great importance to identify and isolate the target antigen(s) located on the larval cuticle. However, our attempts to do so by Western blotting of whole parasite soluble extract, or by immunoprecipitation of surface-labelled proteins, have been unsuccessful. We interpret this as an indication that the critical antigen is not a typical membrane protein,

but is perhaps a glycolipid or else an insolubly cross-linked protein which cannot be broken down into subunits for SDS-PAGE analysis. Insoluble proteins such as the tryosine-bridged cuticlin (Fujimoto & Kanaya 1973) have been reported for adult filariae (Betschart & Jenkins 1987). However, the fact that IgM and IgG2 antibodies were the predominant class/subclass of antibodies made to the L3 surface, indicates that the target epitopes inducing the age-related immune response are more likely to be carbohydrate in nature. Further studies are in progress to define the molecular nature of the determinants involved in induction of the age-specific immune response to the L3 surface.

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