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Immunoepidemiology of *Wuchereria bancrofti* infection in two East African communities: Antibodies to the microfilarial sheath and their role in regulating host microfilaraemia

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ABSTRACT

The response pattern of specific antibodies to the microfilarial sheath (sheath-Ab) of the mosquito-borne filarial parasite *Wuchereria bancrofti* was investigated in individuals from two East African communities with different levels of endemicity. Individuals from both communities presented a strong inverse relationship between positivity for sheath-Ab and being positive for microfilariae (mf) and circulating filarial antigens (CFA). The prevalence of sheath-Ab positivity was highest in young individuals, but peaked at a younger age in the high (1–14 years) than the low (15–19 years) endemicity community. IgG1, IgG2, IgG3 and IgE intensities to a crude adult filarial worm antigen were higher, and IgG4 intensities were lower, in sheath-Ab positive than in sheath-Ab negative individuals, probably reflecting the infection status of individuals. From the study it appears that individuals become sheath-Ab positive before mf and/or CFA can be detected in the peripheral blood, and only after later disappearance of sheath-Ab from the circulation can CFA and mf be diagnosed. In light of the findings, possible roles of the distinct sheath-Ab in the host–parasite relationship are discussed, and a hypothesis is proposed which suggests that sheath-Ab play an important role in the regulation of host microfilaraemia.

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1. Introduction

As early as 1929, it was reported that serum from many amicrofilaraemic individuals from a bancroftian filariasis endemic area contains a factor which can mediate adherence of leucocytes to *Wuchereria bancrofti* microfilariae (mf) in vitro (Pandit et al., 1929). This was later confirmed in other studies (Raghavan et al., 1958; Viswanathan et al., 1973; Subrahmanyam et al., 1978; Simonsen, 1983a, b). The factor responsible for the adherence phenomenon was identified as an antibody which reacted specifically with the microfilarial sheath (Subrahmanyam et al., 1978; Mehta et al., 1981; Simonsen, 1985). The adhering cells were granulocytes, in particular neutrophils but also eosinophils, and the cell adhesion resulted in immobilization and death of the microfilariae (Mehta et al., 1981; Simonsen, 1983b). IgG2 was shown to be the predominant

antibody in this response (Ravindran et al., 1990; Simonsen and Meyrowitsch, 1998), and the main target to be carbohydrate moieties on the microfilarial sheath (Ravindran et al., 1990).

The obvious negative relationship between microfilaraemia and the occurrence of distinct antibodies to the microfilarial sheath (sheath-Ab) in individuals from endemic areas and the capability of these sheath-Ab to mediate cellular attack on microfilariae in vitro, combined with the fact that similar type of antibodies were detected in several animal models of filariasis at the time when the infection progress from the microfilaraemic to the amicrofilaraemic phase (e.g. Wong, 1974; Subrahmanyam et al., 1976; Tanner and Weiss, 1978; Haque et al., 1980; Johnson et al., 1981; Weil et al., 1982), suggested that sheath-Ab were involved in elimination of microfilariae from their hosts. Sheath-Ab thus appeared to play a central role in a protective immunity mechanism in human bancroftian filariasis.

More recent epidemiological observations have, however, raised uncertainty about the role of sheath-Ab in human bancroftian filariasis. First, sheath-Ab were found with highest prevalence

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in children, at an earlier age than microfilaraemia appears in most individuals (Simonsen and Meyrowitsch, 1998; Ravindran et al., 2000). Second, the occurrence of sheath-Ab was not only inversely related to microfilaraemia but also to circulating filarial antigenemia (generally considered a marker of adult worm infection), suggesting that sheath-Ab appear before the maturation of adult worms and their production of microfilariae (Simonsen and Meyrowitsch, 1998; Ravindran et al., 2000). Finally, follow-up studies failed to show that loss of patent microfilaraemia was associated with appearance of sheath-Ab (Simonsen and Meyrowitsch, 1998; Satapathy et al., 2001). These observations from endemic human populations have complicated the analogy to the findings in the animal models, and suggest that sheath-Ab in human bancroftian filariasis are associated with an immunological mechanism expressed with highest prevalence in young individuals before development of patent microfilaraemia. This has even prompted some investigators to suggest that in human bancroftian filariasis, anti-microfilarial immunity is not mediated by sheath-Ab (Ravindran et al., 2003).

Here we investigate the patterns of sheath-Ab positivity from another angle, namely by analysing and comparing the responses in two populations living in communities with markedly different levels of *W. bancrofti* endemicity, as expressed in significant infection, disease and transmission level differences. The relationship of the sheath-Ab to age, microfilaraemia, circulating filarial antigenemia and filarial-specific IgG1, IgG2, IgG3, IgG4 and IgE antibody responses to a crude filarial antigen in the two populations is analysed. The work was carried out as part of a broader study on the immunoepidemiology of *W. bancrofti* infections in coastal East Africa (Michael et al., 2001; Simonsen et al., 2002; Rwegoshora et al., 2005; Jaoko et al., 2006, 2007).

2. Materials and methods

2.1. Study populations and study design

The field work was conducted in 1998 in two communities located approximately 80 km apart within the same East African *W. bancrofti* endemic focus, namely Masaika village in Pangani District (Tanga Region) of Tanzania and Kingwede village in Kwale District (Coast Province) of Kenya. Following informed oral consent to participate (from parents or guardians for those <15 years), individuals aged 1 year and above were examined clinically for filariasis related chronic manifestations and parasitologically for microfilariae, and a venous blood sample was collected for measurement of circulating filarial antigens (CFA) and filarial-specific antibodies. No antifilarial treatment had taken place in the communities prior to sample collection. The study was approved by the Medical Research Coordinating Committee of the National Institute for Medical Research, Tanzania, the Kenyatta National Hospital Ethical and Research Committee, Kenya, and the Central Scientific-Ethical Committee, Denmark.

The study communities have been described elsewhere together with a presentation of findings from the clinical, mf and CFA examinations (Simonsen et al., 2002). Briefly, Masaika and Kingwede had 950 and 1013 inhabitants aged 1 year and above, respectively. In Masaika, overall mf and CFA prevalences were 24.9% and 52.2%, respectively, 4.0% of adults (≥ 20 years) presented with limb elephantiasis, and 25.3% of adult males had hydrocele. In Kingwede, overall mf and CFA prevalences were 2.7% and 16.5%, respectively, 0.9% of adults presented with limb elephantiasis, and 5.3% of adult males had hydrocele. Thus, the endemicity of lymphatic filariasis was obviously higher in Masaika than in Kingwede, a fact also reflected in a much higher level of transmission in the first than the later community (Rwegoshora et al., 2005).

Since measurement of antibodies to the mf sheath is rather laborious and time consuming, the present study was limited to include only individuals from one cluster in Masaika and one cluster in Kingwede, comprising 38% and 33% of the overall populations, respectively. Masaika comprises of four natural clusters of human settlements, and the most western of these (Mlimani) was selected. It had 127 houses with 364 individuals aged ≥ 1 year. The population in Kingwede has a more uniformly scattered settlement pattern, and the south-eastern quarter which had 54 houses and 338 individuals aged ≥ 1 year was selected. With regard to population structure, and filariasis transmission and infection pattern, both clusters were representative for their overall community.

2.2. Clinical and parasitological examination

Villagers ≥ 1 year old were registered in a house-to-house survey shortly before the population was examined. Examinations were carried out as described (Simonsen et al., 2002). Briefly, individuals were clinically examined for signs of lymphatic filariasis related chronic manifestations. Finger-prick blood samples (100 μ l) were collected at night between 21:00 and 24:00 h and examined for *W. bancrofti* mf by the counting chamber method. Venous blood (5 ml) was collected in plain vacutainer tubes immediately thereafter for serum preparation. The serum was later tested for *W. bancrofti*-specific circulating filarial antigens with the TropBio enzyme-linked immunosorbent assay (ELISA) kit (TropBio Pty. Ltd., Townsville, Australia).

2.3. Preparation of serum

Serum was separated by centrifugation after overnight clotting in a fridge, and sodium azide was added to a concentration of 15 mM as a preservative. Serum was initially frozen at -20°C in the field, and later stored at -80°C in the main laboratory until use. Before further handling and testing of sera, lipid coated vira were eliminated by addition of 3 μ l/ml tri-*N*-butyl phosphate (Sigma, T-4908) and 10 μ l/ml Tween 80 (Sigma, P-1754) (Poulsen and Sørensen, 1993).

2.4. Specific antibodies to the mf sheath

Sera were examined for specific antibodies to the mf sheath (sheath-Ab) by an immunofluorescent antibody test (IFAT) using a tube test method with lightly fixed *W. bancrofti* mf in solution, as previously described (Simonsen, 1985). Previous screenings had shown that IgG2 is the predominating antibody type in this response (Ravindran et al., 1990; Simonsen and Meyrowitsch, 1998), and the results presented here are based on IgG2 measurements only. The IFAT reactions were graded according to the brightness of fluorescence (grade 0, I, II and III = no, weak, intermediate and strong fluorescence, respectively), as previously described (Simonsen, 1985). Each specimen was first read by two individuals independently. In case of disagreements in reading, the specimens were re-examined by the same two individuals who thereafter came to a common agreement.

2.5. ELISA measurement of filarial-specific antibodies

Sera were examined for IgG1, IgG2, IgG3, IgG4 and IgE antibodies to a crude filarial antigen (*Brugia pahangi* adult worm homogenate) by enzyme-linked immunosorbent assay, as described previously (Jaoko et al., 2006). Optimal dilutions of antigen, serum and conjugate were determined by titration. The OD value of a positive control serum included on all plates was used to adjust for minor plate-to-plate variation. Prior to measurement of IgE, sera were absorbed

Table 1
Characteristics of the two study populations

	Masaika cluster	Kingwede cluster	P-value ^a
Registered population ≥1 year			
Number	364	338	–
% less than 20 years	47.0	54.7	–
Male: female ratio	1.17	0.87	–
Examined population ≥1 year			
Number (% of registered)	315 (86.5)	247 (73.1)	–
Number mf positive (%)	75 (23.8)	9 (3.6)	<0.001
Number CFA positive (%)	173 (54.9)	30 (12.1)	<0.001
Number sheath-Ab positive (%)	135 (42.9)	144 (58.3)	<0.001
IgG1 mean OD value ^b	1.042	0.549	<0.001
IgG2 mean OD value ^b	0.410	0.126	<0.001
IgG3 mean OD value ^b	0.218	0.917	<0.001
IgG4 mean OD value ^b	0.617	0.100	<0.001
IgE mean OD value ^b	0.303	0.216	<0.001

mf = microfilaria, CFA = circulating filarial antigens, sheath-Ab = antibodies to the mf sheath.

^a Chi-square test for proportions, *t*-test for means.

^b Geometric mean among all examined.

with a protein A agarose bead suspension (Ken-En-Tec A/S, Denmark) at a ratio of 50:140, to remove IgG4 blocking antibodies (Jaoko et al., 2001).

2.6. Data analysis

Geometric mean intensities (GMIs) of circulating filarial antigenemia and filarial-specific antibody levels were calculated as $\text{antilog}[(\sum \log x + 1)/n] - 1$, with *x* the number of CFA units or ELISA OD values, respectively, and *n* the number of individuals included. Proportions were compared by chi-square test and means were compared by independent sample *t*-tests. *P*-values <0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the two study populations

The selected clusters in Masaika and Kingwede had 364 and 338 inhabitants aged ≥1 year, respectively (Table 1). Of these, 47.0% and 54.7%, respectively, were less than 20 years old. The male to female ratio was higher in the Masaika than in the Kingwede cluster mainly because many young adult males had left the latter in search of employment.

Three hundred and fifteen individuals from the Masaika cluster (85.6%) and 247 individuals from the Kingwede cluster (73.1%) were examined for mf, CFA, sheath-Ab by IFAT, and filarial antibodies by ELISA (Tables 1 and 2). Age- and gender-wise, and in relation to mf

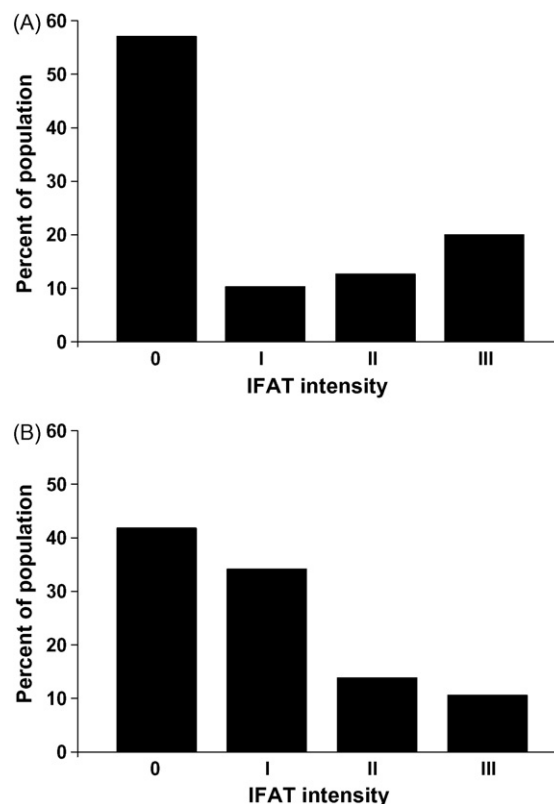


Fig. 1. Percent of population in the Masaika (A) and Kingwede (B) clusters showing different grades of IFAT positivity (sheath-Ab) on the microfilarial sheath (0 = no fluorescence; I to III = increasing brightness of fluorescence).

and CFA, the examined individuals resembled the overall village populations. Prevalences of mf and CFA were significantly higher in the Masaika cluster (23.8% and 54.9%, respectively) than in the Kingwede cluster (3.6% and 12.1%, respectively). On the contrary, the prevalence of positivity for sheath-Ab was significantly higher in the Kingwede cluster (58.3%) than in the Masaika cluster (42.9%). The pattern of distribution of IFAT intensities for the sheath-Ab also differed markedly between the two communities (Fig. 1). Thus, in the Masaika cluster, most sheath-Ab positives were grade III (46.7%) followed by grade II (29.6%) and grade I (23.7%). In the Kingwede cluster, most sheath-Ab positives were grade I (58.3%) followed by grade II (23.6%) and grade III (18.1%). Mean ELISA OD values for IgG1, IgG2, IgG4 and IgE were significantly higher in the Masaika cluster than in the Kingwede cluster, while the opposite was seen for IgG3 (Table 1).

Table 2
Study population size, *Wuchereria bancrofti* microfilariemia and antigenemia, and sheath antibody status in relation to age group in the Masaika and Kingwede clusters

Age group (years)	Masaika cluster				Kingwede cluster			
	Number examined individuals	Number mf positive (%)	Number CFA positive (%)	Number sheath-Ab positive (%)	Number examined individuals	Number mf positive (%)	Number CFA positive (%)	Number sheath-Ab positive (%)
1–4	28	0 (0.0)	4 (14.3)	15 (53.6)	35	0 (0.0)	0 (0.0)	13 (37.1)
5–9	38	4 (10.5)	15 (39.5)	19 (50.0)	34	0 (0.0)	1 (2.9)	19 (55.9)
10–14	40	7 (17.5)	21 (52.5)	25 (62.5)	47	0 (0.0)	5 (10.6)	34 (72.3)
15–19	37	12 (32.4)	20 (54.1)	16 (43.2)	22	1 (4.5)	1 (4.5)	17 (77.3)
20–29	57	16 (28.1)	35 (61.4)	24 (42.1)	50	3 (6.0)	10 (20.0)	31 (62.0)
30–39	48	11 (22.9)	27 (56.3)	19 (39.6)	27	2 (7.4)	5 (18.5)	18 (66.7)
40–49	34	9 (26.5)	25 (73.5)	10 (29.4)	7	1 (14.3)	3 (42.9)	3 (42.9)
50+	33	16 (48.5)	26 (78.8)	7 (21.2)	25	2 (8.0)	5 (20.0)	9 (36.0)
All	315	75 (23.8)	173 (54.9)	135 (42.9)	247	9 (3.6)	30 (12.1)	144 (58.3)

mf = microfilaria, CFA = circulating filarial antigens, sheath-Ab = antibodies to the mf sheath.

Table 3

The relation of microfilaraemia, circulating filarial antigenemia and ELISA antibody response to sheath antibody status in the Masaika and Kingwede clusters

	Masaika cluster			Kingwede cluster		
	Negative for sheath-Ab	Positive for sheath-Ab	P-value ^a	Negative for sheath-Ab	Positive for sheath-Ab	P-value ^a
Number in category	180	135	–	103	144	–
Number mf positive (%)	75 (41.7)	0 (0.0)	–	9 (8.7)	0 (0.0)	–
Number CFA positive (%)	154 (85.6)	19 (14.1) ^d	<0.001	28 (27.2)	2 (1.4) ^e	–
CFA mean antigen units ^b	7356	262	<0.001	999	49.8	–
IgG1 mean OD value ^c	0.900	1.242	<0.001	0.480	0.602	=0.02
IgG2 mean OD value ^c	0.318	0.542	<0.001	0.105	0.142	>0.05
IgG3 mean OD value ^c	0.152	0.313	<0.001	0.757	1.040	=0.01
IgG4 mean OD value ^c	0.781	0.421	<0.001	0.126	0.082	>0.05
IgE mean OD value ^c	0.272	0.345	=0.005	0.159	0.259	<0.001

mf = microfilaria, CFA = circulating filarial antigens, sheath-Ab = antibodies to the mf sheath.

^a Chi-square test for proportions, *t*-test for means.^b Geometric mean among positives only.^c Geometric mean among all examined.^d 12 grade I, 5 grade II, 2 grade III (mean grade for 116 CFA negative = 2.35; for 19 CFA positive = 1.47; *P* < 0.001).^e Both grade I (mean grade for 142 CFA negative = 1.61; for 2 CFA positive = 1.00).

3.2. Relation of sheath-Ab to microfilaraemia, circulating filarial antigenemia and ELISA antibody responses

In both clusters, there was a strong negative relationship between being positive for sheath-Ab and microfilaraemic. Thus, all sheath-Ab positive individuals were mf negative, and all mf positive individuals were negative for sheath-Ab (Table 3). Among the sheath-Ab negative individuals, 41.7% and 8.7% were mf positive in the Masaika and Kingwede cluster, respectively.

By far the majority of CFA positive individuals in both clusters were negative for sheath-Ab (89.0% and 93.3% for Masaika and Kingwede, respectively), resulting in a significantly higher CFA prevalence in sheath-Ab negative than in the sheath-Ab positive individuals in both clusters (Table 3). Among those being positive for both CFA and sheath-Ab, the CFA intensity was significantly lower than among those being positive for CFA but negative for sheath-Ab, and most had low grade IFAT positivity.

In the Masaika cluster, the mean IgG1, IgG2, IgG3 and IgE ELISA OD values were significantly lower and the mean IgG4 values significantly higher in the individuals negative than among individuals positive for sheath-Ab (Table 3). In the Kingwede cluster similar significant differences were observed for IgG1, IgG3 and IgE, whereas differences for IgG2 and IgG4 were not significant. Mean ELISA OD values in both communities were generally lower among individuals negative for both sheath-Ab and CFA than among individuals positive for one or both of these responses. This is illustrated for IgG4 in Table 4, both for all age groups combined, and after excluding the two youngest age groups (which contained a high proportion of the individuals).

3.3. Relation of sheath-Ab to age and infection in the two communities

The age-distribution of positivity for sheath-Ab is shown in Table 2 and Fig. 2. In the Masaika cluster, the highest prevalence was in the young individuals. This was followed by a decrease in prevalence with age. The prevalence in the 1–14 years age group was significantly higher than in 15+ years age group (55.7% vs. 36.4%; *P* = 0.001). In the Kingwede cluster, the prevalence increased by age among children to a peak in the 15–19 years age group (77.3%) and it thereafter decreased by age. The prevalence in the 10–19 years age group (73.9%) was significantly higher than the prevalence in the 1–9 years age group (46.4%; *P* = 0.001) and the 20+ years age group (56.0%; *P* = 0.016). The age-distribution of the different grades of positivity generally followed the overall pattern of distribution in both communities.

Fig. 3 shows simultaneously the age-distribution of positivity for sheath-Ab and CFA by age group in the two communities. In both clusters, many children in the youngest age group had sheath-Ab (higher prevalence in Masaika than in Kingwede), whereas few (Masaika) or none (Kingwede) had CFA. In the Masaika cluster there was a rapid increase in CFA prevalence by age, and in the 10–14 years age group almost everybody had either sheath-Ab, CFA or both. In subsequent years the decrease in prevalence of sheath-Ab was compensated for by a further increase in CFA prevalence, thus maintaining almost complete age group coverage with either of these two responses. In the Kingwede cluster the CFA prevalence rose only slowly by age, and despite the higher prevalences of sheath-Ab, the combined responses never reached as high levels as in Masaika. A marked decrease in CFA preva-

Table 4

Mean ELISA OD values for IgG4 in relation to individual's response status for antibodies to the microfilarial sheath (sheath-Ab) and circulating filarial antigens (CFA) in the Masaika and Kingwede clusters

Response status group	IgG4 mean OD value ^a			
	Masaika cluster		Kingwede cluster	
	≥ 1 year	≥ 10 years	≥ 1 year	≥ 10 years
Sheath-Ab neg/CFA neg	0.123 (<i>n</i> = 26)	0.273 (<i>n</i> = 10)	0.036 (<i>n</i> = 75)	0.060 (<i>n</i> = 39)
Sheath-Ab pos/CFA neg	0.352 (<i>n</i> = 116)	0.329 (<i>n</i> = 85)	0.078 (<i>n</i> = 142)	0.092 (<i>n</i> = 110)
Sheath-Ab pos/CFA pos	0.926 (<i>n</i> = 19)	0.942 (<i>n</i> = 16)	0.435 (<i>n</i> = 2)	0.435 (<i>n</i> = 2)
Sheath-Ab neg/CFA pos	0.925 (<i>n</i> = 154)	0.925 (<i>n</i> = 138)	0.405 (<i>n</i> = 28)	0.400 (<i>n</i> = 27)

n = number of individuals in group, pos = positive, neg = negative.^a Geometric mean among all examined.

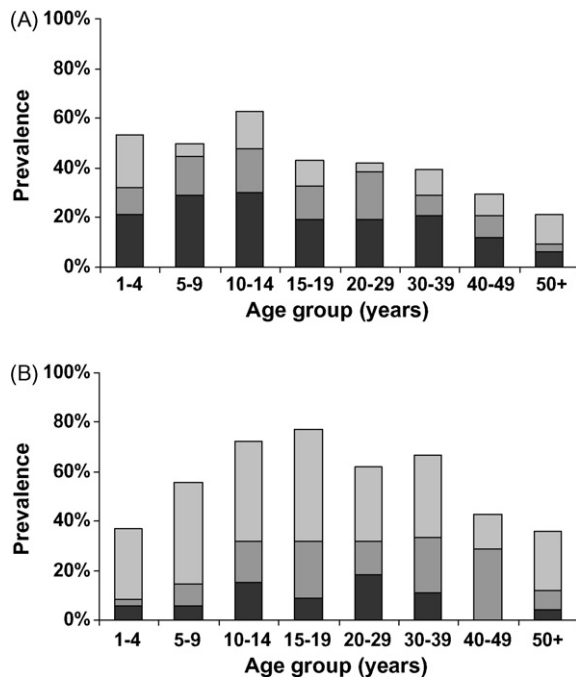


Fig. 2. Age-distribution of the different grades of IFAT positivity (sheath-Ab) on the microfilarial sheath in the Masaika (A) and Kingwede (B) clusters. (Light grey part of bar = weak (I); medium grey part of bar = medium (II); dark grey part of bar = strong (III)).

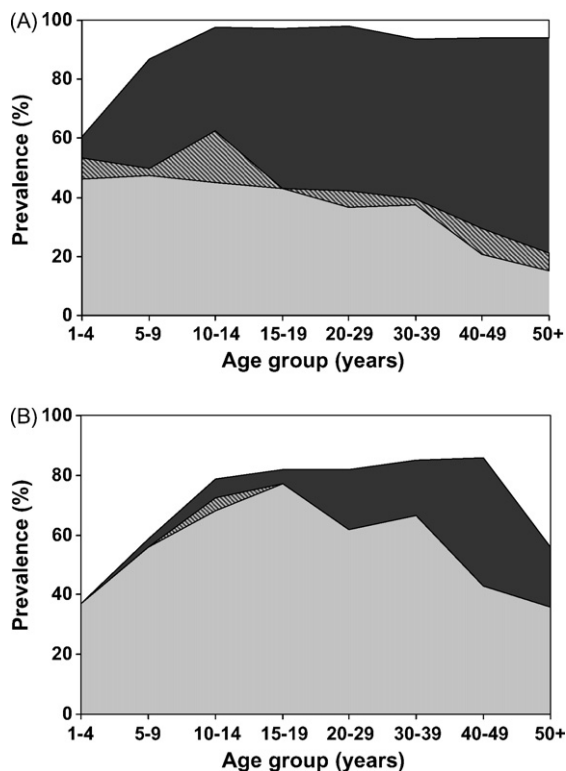


Fig. 3. Age-distribution of positivity for sheath-Ab (IFAT positivity) and *W. bancrofti* infection (CFA positivity) in the Masaika (A) and Kingwede (B) clusters. Grey area = sheath-Ab positive only; hatched area = sheath-Ab + CFA positive; black area = CFA positive only; white area = negative for both sheath-Ab and CFA.

lence was observed in the oldest age group in the Kingwede cluster.

4. Discussion

The infection, disease and transmission characteristics reported earlier from Masaika and Kingwede (Simonsen et al., 2002; Rwegoshora et al., 2005), with significantly higher levels in the former than the latter, were clearly reflected in the community clusters selected for the present study. Two different techniques were used for detecting the filarial antibody responses. First, ELISAs were performed with an adult *B. pahangi* homogenate as the antigen. The measured OD responses to this antigenic mixture reflect human sensitization to a variety of filarial antigens of mainly proteinaceous nature. The ELISA antibody responses have been reported and analysed in detail before for these two communities (Jaoko et al., 2006, 2007), and the response patterns observed in the selected community clusters resembled those observed for the overall communities. Second, IFATs were performed with whole lightly fixed *W. bancrofti* mf. This technique measures specific antibodies to the mf sheath (Simonsen, 1985). The antibodies are primarily of the IgG2 isotype and have high affinity for carbohydrate moieties in the mf sheath (Ravindran et al., 1990; Simonsen and Meyrowitsch, 1998). Since the two techniques recognize markedly different types of antigens it is not surprising that the IgG2 ELISA OD values showed no relationship to the IgG2 IFAT measurements.

When ELISA OD values of sheath-Ab positive and negative individuals were compared, the response patterns closely resembled those observed in CFA negative and CFA positive individuals, respectively (Jaoko et al., 2006). This is probably a reflection of the inverse relationship between sheath-Ab and CFA status. The generally low levels of OD values observed in individuals being negative for both sheath-Ab and CFA could suggest that these had been less exposed to infection than the remaining part of the population. Filarial-specific IgG4 responses correlate well with the presence and duration of active infection (Kwan-Lim et al., 1990; Haarbrink et al., 1995). In this respect it was interesting in the present study to note that the lowest level of IgG4 was in the sheath-Ab/CFA negative group. This was followed by a higher level in the sheath-Ab positive/CFA negative group, and the highest level in the CFA positive groups.

There was a strong inverse relationship between sheath-Ab positivity and the two markers of filarial infection (mf and CFA). This was particularly evident for microfilaremia, with all mf positive individuals being sheath-Ab negative and visa versa. A similar relationship has been noted before, in bancroftian filariasis (e.g. Subrahmanyam et al., 1978; Simonsen, 1983a; Ravindran et al., 2000), brugian filariasis (McGreevy et al., 1980), onchocerciasis (Greene et al., 1981) and loiasis (Pinder et al., 1992) in humans and in several laboratory models of filariasis (e.g. Wong, 1974; Subrahmanyam et al., 1976; Tanner and Weiss, 1978; Johnson et al., 1981; Weil et al., 1982), and has been taken as an indication that the antibodies are involved in a host defense mechanism directed against the mf. The high levels of stage and species specificity of the responses (Weiss and Tanner, 1981; Wong, 1974; Haque et al., 1980; Simonsen, 1983b; Thompson et al., 1985) also suggest that they play a key role in regulating parasite density in the host. CFA are generally regarded as markers of adult worm infection (Nicolas, 1997), and in agreement with earlier studies (Simonsen and Meyrowitsch, 1998; Ravindran et al., 2000), the majority of sheath-Ab positive individuals were negative for CFA. However, a few of the sheath-Ab positive individuals were simultaneously positive for CFA, namely 14% in Masaika and 1% in Kingwede. Among these, the mean IFAT grades and the mean CFA levels were significantly lower than among those positive for only one of the

responses, and it is possible that the ‘double-positive’ individuals represent a stage of transition from being positive for one to being positive for the other response.

The study provides some evidence for the developmental sequence of sheath-Ab in relation to the appearance of the filarial infection markers in the peripheral blood. First, the high prevalence of sheath-Ab in the youngest age group in both communities, before the age of appearance of cases of microfilaraemia, suggests, in agreement with earlier studies (Simonsen and Meyrowitsch, 1998; Ravindran et al., 2000), that stimulation of sheath-Ab production takes place before mf are detected in the peripheral blood. Second, the fact that the youngest age groups had few or no CFA positive cases suggests that sheath-Ab also appear before release of measurable amounts of CFA to the circulation. Finally, the decrease in sheath-Ab prevalence with increasing age in the adult human populations in both communities was accompanied by a simultaneous increase in prevalence of mf and CFA. In summary, the study indicates that exposed individuals first become sheath-Ab positive, and only after down-regulation of the sheath-Ab response can mf and/or CFA be detected in the peripheral blood. The study gave no evidence that individuals subsequently clear the infection and become sheath-Ab positive, as observed in the animal models. The outlined sequence is in agreement observations from longitudinal studies, which have failed to detect sheath-Ab in individuals after loss of patent *W. bancrofti* microfilaraemia (Simonsen and Meyrowitsch, 1998; Satapathy et al., 2001).

The response pattern of sheath-Ab differed between the high and low endemicity community, most likely because of the higher transmission intensity in the former than the latter. The peak prevalence of sheath-Ab positivity was thus seen in a younger age group in Masaika than in Kingwede. Overall, the individual sheath-Ab responses were also stronger in Masaika than in Kingwede, and probably for this reason the down-regulation of sheath-Ab and transformation CFA positivity occurred at an earlier age. The high overall prevalence of sheath-Ab positivity in Kingwede, but low overall intensity, suggests that most individuals in Kingwede were exposed, but that down-regulation of sheath-Ab and transformation to CFA positivity, was delayed.

Two intriguing questions are obviously pressing, namely (1) what induces the sheath-Ab, and (2) what role do they play in the host–parasite relationship? When considering that individuals appear to become sheath-Ab positive before they become CFA (and mf) positive, the most straightforward interpretation would be that sheath-Ab production is stimulated by exposure to L3s or to early developing larval stages and that these antibodies cross react with the mf sheath (Ravindran et al., 2003). Later, when the adult worms mature, the production is down-regulated, and the sheath-Ab play no role in the regulation of the mf population (Ravindran et al., 2003). It would be surprising, however, if this distinct response, observed in all species of filaria studied, which in vitro is capable of mediating cellular attack and cytotoxicity, and the appearance of which in several animal models correlate well with disappearance of mf, should be just an artefact in human bancroftian filariasis. What are possible alternatives?

One element of uncertainty when trying to figure out the regulation and role of sheath-Ab in bancroftian filariasis is the limited knowledge about the dynamics of secretion of CFA, i.e. what is actually measured with the CFA test. Studies on *Dirofilaria immitis* have shown that CFA are found most abundantly in the intrauterine ground substance of mature adult female worms, and that CFA are released by the females in vitro in relation to production of mf (Weil and Liftis, 1987). This agrees well with the fact that generally all mf positive individuals (in addition to some mf negative individuals) in bancroftian filariasis are positive for CFA. It might be speculated that the mature adult female worms initiate the repro-

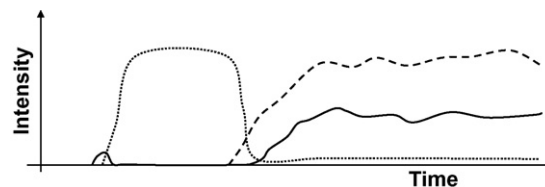


Fig. 4. Schematic presentation of the outlined hypothesis for the sequence of release of microfilariae (mf), sheath-Ab and circulating filarial antigens (CFA) to the peripheral circulation in *W. bancrofti* infections. Infection takes place at Time 0. Following a pre-patent period of development, few mf are released from the adult female worm. These induce a strong sheath-Ab response which prevents further production and/or release of mf and CFA to the circulation. Later, down-regulation of the sheath-Ab response (perhaps due to continued exposure) to levels not measurable with the employed method allows the release of CFA and mf. Full line = microfilaria intensity; dotted line = sheath-Ab intensity; Dashed line = CFA intensity.

ductive career by releasing a few mf in the yet CFA negative host (Fig. 4). These may induce a strong antibody response to the mf sheath (as seen after injection of live mf in experimental animals: Wong, 1964; Bagai and Subrahmanyam, 1970; Thompson et al., 1981, 1985; Zahner and Wegerhof, 1985), which then for a considerable period of time prevents further mf production and/or release, as well as prevents production and/or release of CFA (the sheath-Ab positive, but CFA/mf negative, period). This would also explain the generally observed low mf prevalence in young children, even in areas of heavy transmission. As time goes, the sheath-Ab response is down-regulated (perhaps due to continued exposure to infective larvae) after which CFA and mf may be released and become measurable in the peripheral blood. It may be further speculated that during the patent (mf positive) period, low levels of sheath-Ab are still released as a mechanism for regulation of mf intensity levels (Wong, 1964), which have been observed to be extraordinary stable for long periods in microfilaraemic individuals.

An implication of this hypothesis would be that not only CFA positive individuals but also sheath-Ab positive individuals carry adult filarial worms. This would give a considerable increase adult worm infection prevalence (to 91.7% in Masaika and 69.6% in Kingwede) when compared to the situation when only those positive for CFA are considered adult worm positive (54.9% and 12.1%, respectively). In combination with the apparent effect of transmission intensity on regulation of sheath-Ab, this hypothesis would furthermore imply that intervention measures aiming at reducing transmission intensity would lead to a prolonged sheath-Ab positive period and a reduced rate of transformation to CFA (and mf) positivity.

Another route by which the hosts' immune system could be sensitized to mf before development of mature adult worms is through the passing of mf (or mf antigens) from the pregnant mother to the foetus. Mf of *W. bancrofti* have been observed to cross the placenta (Eberhard et al., 1993), and there is evidence that infection in pregnant women may affect the course of infection of the child later in life (Lammie et al., 1991). Mf crossing the placenta have also been observed to occur commonly in experimental filariasis (Aniwatangkoo et al., 1996). However, the fact that the prevalence of sheath-Ab positivity in Kingwede still increased up to the 15–19 years age group, and that even in Masaika the prevalences of CFA in the older age groups were higher than those of sheath-Ab positivity in the youngest age group, seems to indicate that mother–child sensitization cannot be the only cause of a high sheath-Ab prevalence in the children. CFA can also cross the placenta (Weerasinghe et al., 2005) and may contribute to the CFA positivity observed, in particular in Masaika, in younger age groups.

The host–parasite relationship in bancroftian filariasis is complex and many aspects are not yet properly understood. Further defining the significance of the rather distinct and specific

responses of sheath-Ab production by the host and CFA release from the parasites, both of which appear to reflect specific phases in the host–parasite relationship, may help in paving the way forward. In particular, the relation of CFA release and measurability to parasite biology, and the role of sheath-Ab as a host regulatory response, should be further looked into. A better understanding of the regulatory processes involved in the host–filaria relationship may help in the combat against the major public health problem caused by filarial parasites in large parts of the developing world.

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