

BANCROFTIAN FILARIASIS IN TANZANIA: SPECIFIC ANTIBODY RESPONSES IN RELATION TO LONG-TERM OBSERVATIONS ON MICROFILAREMIA

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Abstract. Following a 16-year clinical and parasitologic follow-up survey for Bancroftian filariasis in three endemic communities in northeastern Tanzania, serum antibody responses were analyzed in selected individuals in relation to the long-term observations on microfilaremia. Comparison of responses in three categories of adults (microfilaria [mf] positive at both surveys, mf positive at first but mf negative at the second survey, and mf negative at both surveys, respectively) indicated no significant differences between the mean levels of filarial-specific IgG1, IgG2, IgG3, IgG4, or IgE (measured by ELISA). However, specific IgG2 to the sheath of *Wuchereria bancrofti* mf (measured by an indirect fluorescence antibody test [IFAT]) was detected only in the third category. Comparison of responses in two categories of children born around the time of the first survey (to mf-positive and mf-negative mothers, respectively) showed a significantly higher mean level of filarial-specific IgG4 in the first than in the latter category, whereas the mean levels of filarial-specific IgG1, IgG2, IgG3, and IgE, and the prevalences of IgG2 IFAT positivity were similar. The overall prevalence of IgG2 IFAT positivity was considerably higher in the child study population (45.5%) than in the adult study population (16.7%). In both populations, however, a clear association between IgG2 IFAT positivity and a negative microfilarial status and negative specific circulating antigen status was seen. The study suggests that specific anti-sheath-antibodies are associated with an immunologic resistance mechanism that in the endemic community is expressed with highest prevalence in young individuals before development of patent microfilaremia.

Despite the widespread occurrence of *Wuchereria bancrofti* infections in tropical areas, many aspects of the natural history of infection and disease in Bancroftian filariasis remain poorly understood. Basic knowledge on determinants related to host susceptibility, the longevity of infection and development of resistance, being of utmost importance for understanding the epidemiology of Bancroftian filariasis and for planning and implementation of control programs, is lacking.^{1–3} A major reason for this is a scarcity of longitudinal studies.

In an attempt to clarify some of these aspects, a 16-year follow-up study was carried out on Bancroftian filariasis in three endemic communities in northeastern Tanzania. All individuals were examined for microfilariae and clinical manifestations in 1975⁴ and again in late 1991,⁵ and as many as possible of the individuals examined in the first surveys were re-identified in the second surveys. Analyses of the parasitologic and clinical findings have already been presented.^{5,6} Briefly, with respect to microfilaremia, the study showed that only few individuals had changed from a microfilaria (mf)-positive to -negative status over the 16-year period, despite the fact that the average life span of adult worms has been estimated to be much shorter.¹ In the 1991 surveys, it was furthermore observed that in children 1–15 years of age, the microfilarial prevalence was considerably higher among those with microfilaremic mothers than among those with amicrofilaremic mothers, whereas it had no relationship to the fathers mf status, thus confirming previous similar observations from Haiti.⁷ These sets of observations strongly indicate that some individuals in the endemic community are more susceptible to develop microfilaremia than others, and that once microfilaremia has been acquired it persists for a very long time.

Shortly after the second survey (in early 1992), individuals were selected from the same communities for further investigations on their humoral immune responses to filarial antigen. The aim was to see if these responses showed any

relationship to the long-term observations on mf status. Here, two studies will be presented. The first examined antibody responses in individuals in relation to their microfilarial status in 1975 and 1991. It included individuals who were mf positive at both surveys, individuals who were mf positive in 1975 but mf negative in 1991, and individuals who were mf negative at both surveys. The second examined the antibody responses in young individuals (10–20 years of age in 1991) in relation to the mf status of their mother around the time of her pregnancy. Two types of tests were performed on the sera for measurement of antibodies. First, an ELISA (for IgG1, IgG2, IgG3, IgG4, and IgE) was used as a general test for antibody responsiveness to filarial antigen. Second, an indirect fluorescence antibody test (IFAT) was used for measurement of specific antibodies to the sheath of *W. bancrofti* microfilariae. These antibodies have previously been associated with a resistance mechanism in Bancroftian filariasis.^{8,9}

MATERIALS AND METHODS

Study populations and study design. The study individuals were from three coastal villages (Machui, Kwale, and Tawalani) in the Tanga Region of northeastern Tanzania. The villages were surveyed parasitologically and clinically for Bancroftian filariasis in 1975⁴ and again in late 1991.⁵ During the 1991 surveys, individuals who participated in the 1975 surveys were re-identified to analyze at an individual level the development of microfilaremia and clinical manifestations over the 16-year period.⁶ Based on the results from these surveys, two studies were designed to assess the immunologic responsiveness of selected individuals in relation to the long-term observations on mf status. Informed oral consent to participate was obtained from all adults and from parents of children less than 15 years of age. The study was reviewed and approved by the Central Scientific-Ethical

Committee of Denmark and by the Tanzania Commission for Science and Technology.

The first study (Study 1) examined antibody responses in adult individuals (21–80 years of age in 1991, mean age = 47 years) in relation to their microfilarial status in 1975 and 1991. Forty-eight individuals whose mf status was determined at both surveys were included (Table 1). They belonged to three categories: 20 were mf positive at both surveys, 13 were mf positive in 1975 but mf negative in 1991, and 15 were mf negative at both surveys. Three males in the first category and one in the second category had hydrocele, whereas no individuals showed signs of lymphoedema/elephantiasis.

The second study (Study 2) examined antibody responses in young individuals/children (10–20 years of age in 1991, mean age = 14 years) in relation to the mf status of their mothers 16 years previously, i.e., around the time of the mother's pregnancy. There were eight children born to mf-positive mothers and 14 children born to mf-negative mothers. One child in each category was mf positive at the time of blood sampling. Only one mother had changed mf status between the two surveys, namely from negative to positive. None of the children had clinical manifestations related to Bancroftian filariasis.

Parasitologic and clinical examinations. Parasitologic and clinical examinations were performed as previously described.^{5,6} Briefly, 100 µl of fingerprick blood collected between 9:00 PM and 1:00 AM was examined for mf using the counting chamber technique.¹⁰ Clinical manifestations of hydrocele and lymphoedema/elephantiasis were graded according to size and developmental stage.

Serology. Shortly after the clinical and parasitologic examinations, and before any mass chemotherapy was given, venous blood samples were collected for serology (in early 1992). After clotting overnight in a refrigerator, serum was separated from the clot by centrifugation. Sodium azide was added to a concentration of 15 mM as a preservative, and the serum was stored at –80°C until use.

The sera were examined for *W. bancrofti*-specific circulating antigens (CIA) by the Og4C3 TropBio kit (JCU Tropical Biotechnology, Queensland, Australia). The ELISA was performed on serum after boiling pretreatment, according to instructions from JCU Tropical Biotechnology. Samples were tested in duplicate and the mean optical density (OD) values were calculated. Samples with a mean OD value \geq standard 2 (40 antigen units), i.e., samples belonging to titer group 3 and above, were considered antigen positive.

Filarial-specific IgG1, IgG4, and IgE antibodies were measured by ELISA as previously described.¹¹ A similar ELISA procedure was used for measuring filarial-specific IgG2 and IgG3 antibodies, with sera diluted 1:500 for both isotypes, and horseradish peroxidase-conjugated mouse anti-human IgG2 and IgG3 monoclonal antibodies (Jansen, Belgium) used as capture antibodies at 1:2,000 and 1:1,000 dilutions, respectively. A *Brugia pahangi* adult worm homogenate was used as antigen.¹¹ All tests were carried out in triplicate, and the mean OD values were calculated.

The sera were also tested for specific antibodies to the microfilarial sheath by an IFAT using a tube test method with lightly fixed *W. bancrofti* mf in solution as previously described.⁹ Preliminary screenings using fluorescein isothio-

TABLE 1
Baseline information and response patterns for adults in relation to their microfilaria (mf) status in 1975 and 1991 (Study 1)*

Category	Mf status 1975/1991	No. of individuals (males/females)	Mean age in years in 1991 (range)	No. CIA positive (%)	Mean CIA level†	Mean ELISA OD value					No. IFAT positive (%)
						IgG1	IgG2	IgG3	IgG4	IgE	
1	Pos/pos	20 (12/8)	44.2 (33–65)	20 (100)	7.4	194.9	173.8	169.5	449.6	45.2	0
2	Pos/neg	13 (6/7)	52.8 (21–80)	11 (85)	5.2	183.3	255.4	318.0	241.1	92.3	0
3	Neg/neg	15 (6/9)	44.3 (30–55)	7 (47)	4.9	256.3	226.1	224.7	352.4	97.5	8 (53)

* CIA = circulating antigens; OD = optical density; IFAT = indirect fluorescence antibody test; Pos = positive; Neg = negative.
† Mean of titer group among CIA-positive individuals.

cyanate-labeled mouse anti-human IgG1, IgG2, IgG3, and IgG4 monoclonal antibodies (Sigma, St. Louis, MO) and goat anti-human IgM and IgE polyclonal antibodies (Sigma) showed that IgG2 was the predominating antibody type in this response (unpublished data), and the results presented here are based on IgG2 measurements only. The IFAT reactions were graded according to the brightness of fluorescence, as previously described.⁹

Data analysis. Proportions were compared by chi-square tests and means were compared by the Mann-Whitney U-test and the Kruskal-Wallis test (as appropriate). Any P values < 0.05 were considered statistically significant.

RESULTS

Response patterns in adults in relation to their mf status in 1975 and 1991 (Study 1). Table 1 shows the results from the first study in relation to study category. As expected, all individuals in category 1 (mf positive at both surveys) were CIA positive, whereas only some individuals in categories 2 and 3 (mf negative in the latter survey) were CIA positive. The frequency of CIA positivity as well as the mean level of CIA among CIA positive individuals varied significantly between the three categories ($P < 0.001$ by chi-square test and $P < 0.01$, by Kruskal-Wallis test, respectively), with both being highest in category 1 and lowest in category 3. Two individuals in category 2 had changed from being mf positive in 1975 to being both mf negative and CIA negative in 1992, whereas the remaining 11 individuals in this category were still CIA positive (and therefore probably had cryptic adult worm infections or very low level microfilaremias), although they were diagnosed as mf negative.

There were no statistically significant differences among the three study categories for any of the specific antibodies (IgG1, IgG2, IgG3, IgG4, and IgE) measured by ELISA ($P > 0.05$, by Kruskal-Wallis test). However, the mean OD values for IgG4 were higher and those for IgG1, IgG2, IgG3, and IgE were lower among those who were mf positive in 1992 (category 1) than in those who were mf negative (categories 2 and 3 combined). For IgG4, this difference was statistically significant ($P < 0.05$, by Mann-Whitney U test). When analyzing ELISA OD values in relation to CIA status, those for IgG4 were significantly higher among CIA-positive than among CIA negative-individuals (mean = 0.424 versus 0.131; $P < 0.01$, by Mann-Whitney U test), whereas no significant relationship to CIA status was seen for IgG1, IgG2, IgG3, or IgE.

The IgG2 antibodies against the *W. bancrofti* sheath were detected by the IFAT in sera from eight individuals (16.7% of the study population). It was remarkable, however, that all IFAT-positive individuals were in category 3 (those who were mf negative at both surveys), giving a prevalence of 53.3% for IFAT positivity in this category, whereas individuals in categories 1 and 2 were all IFAT negative (Table 1). As shown in Figure 1, the prevalence of IFAT positivity in category 3 was considerably higher among CIA-negative (87.5%) than among CIA-positive (14.3%) individuals, and the difference was statistically significant ($P < 0.01$, by chi-square test). Statistical analysis of the data from category 3 indicated no relationship between IgG1, IgG2, IgG3, IgG4,

or IgE ELISA OD values and the IFAT status of the study individuals.

Response patterns in children in relation to the mf status of their mother around the time of her pregnancy (Study 2). The results from the second study are shown in Table 2. There were no significant difference in circulating antigen pattern between children born to microfilaremic and amicrofilaremic mothers either in prevalence of CIA positivity (50% versus 57%; $P > 0.05$, by chi-square test) or in mean levels of CIA among CIA-positive individuals (6.3 versus 5.3; $P > 0.05$, by Mann-Whitney U test).

The mean ELISA OD value for IgG4 was statistically significantly higher in children born to mothers who were mf positive than in those who were mf negative in 1975 ($P < 0.05$, by Mann-Whitney U test). No differences were seen in mean IgG1, IgG2, IgG3, or IgE ELISA OD values between these two categories. Furthermore, there were no statistically significant differences in IgG1, IgG2, IgG3, IgG4, or IgE ELISA OD values between CIA-positive and CIA-negative individuals. Similar conclusions were reached when the two mf-positive children were excluded from the statistical analyses.

Antibodies against the mf sheath were detected by IFAT in 45.5% of the study population, and with approximately similar frequencies in sera from children of categories 1 and 2 (50% versus 43%; $P > 0.05$, by chi-square test). However, it was obvious for both categories that IFAT positivity was much more common in CIA-negative than in CIA positive children (Figure 1). Overall, 90% (9 of 10) of the CIA-negative children were IFAT positive, whereas only 8.3% (1 of 12) of the CIA-positive children were IFAT positive ($P < 0.001$, by chi-square test). No statistically significant differences in ELISA OD values were observed for IgG1, IgG2, IgG3, IgG4, or IgE between IFAT-positive and IFAT-negative children. Again, similar conclusions were reached when the two mf-positive children were excluded.

DISCUSSION

The basis for the present study was two cross-sectional parasitologic and clinical surveys for Bancroftian filariasis carried out in highly endemic communities over a 16-year interval.⁴⁻⁶ During the second survey, it was particularly emphasized, through repeated rounds of detailed interviews, to re-identify individuals who had been examined at the first survey, and to establish the identity of parents of children 1-20 years of age. Venous blood for antibody measurements were collected from selected individuals after the second survey. The availability of serum furthermore allowed examination for specific *W. bancrofti* circulating antigens with the Og4C3 test, as an additional tool to determine infection status. This test is now considered highly specific and more sensitive than detection of microfilaremia for diagnosis of infection,^{12,13} and CIA positivity in the absence of microfilaremia is generally considered to be due to cryptic adult worm infections or ultra-low levels of microfilaremia.

Study 1 was designed to look for associations between mf status, as observed over the 16-year period, and specific antibody responses. A significant response difference between the three categories would be suggestive of the involvement of the measured response in determining the individuals' mi-

TABLE 2
Baseline information and response patterns for children in relation to microfilaria (mf) status of their mother around the time of her pregnancy (Study 2)*

Cate- gory	Mf status of mother in 1975	No. of children (males/females)	Mean age in years in 1991 (range)	No. CIA positive (%)	Mean CIA level†	Mean ELISA OD value					No. IFAT positive (%)
						IgG1	IgG2	IgG3	IgG4	IgE	
1	Pos	8 (5/3)	14.8 (10-20)	4 (50)	6.3	639.5	463.3	172.6	927.6	225.0	4 (50)
2	Neg	14 (7/7)	13.8 (11-16)	8 (57)	5.3	637.6	369.8	183.4	555.4	262.9	6 (43)

* For definitions of abbreviations, see Table 1.

† Mean of titer group among CIA-positive individuals.

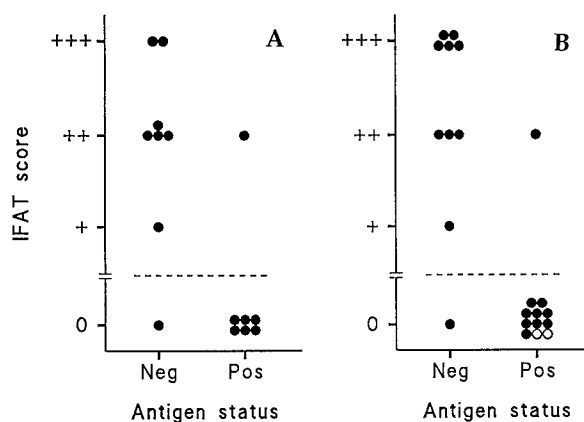


FIGURE 1. Indirect fluorescence antibody test (IFAT) (IgG2) status in relation to circulating antigen status in Study 1 (A, category 3 only) and Study 2 (B). The IFAT results were graded according to brightness of fluorescence. Closed circles indicate microfilaria (mf)-negative individuals; open circles indicate mf-positive individuals. The dashed lines indicate the cut-off values between negative and positive IFAT responses. Neg = negative; Pos = positive.

crofilarial category status. Further characterization of the study individuals in relation to their specific circulating antigens status indicated, as expected,¹³ that all mf-positive and many of the mf-negative individuals were CIA positive. In category 2, however, only two of the 13 adults who had changed from mf-positive to mf-negative status over the 16-year period were CIA negative in the latter survey. Thus, only two individuals appeared to have cleared their infection completely. Combining the results of the low microfilarial loss rate seen over the 16-year period⁶ with the observed low CIA-positive to -negative conversion rate in those who had cleared their microfilaremia indicates that complete clearing of an infection with *W. bancrofti* must be an extremely rare event. This conclusion is in agreement with the continued increase in prevalence of CIA positivity with age seen in population-based studies.^{13,14}

Study 2 examined for associations between mothers' microfilarial status around the time of pregnancy and their children's subsequent specific antibody responses after years of exposure to filarial infection. Considerable differences in mf prevalences had been observed between children born to mf-positive mothers and those born to mf-negative mothers in these communities,⁵ which could be a result of prenatal immunologic sensitization of children born to infected mothers. Microfilaria prevalences are generally low in children, and only two of those selected for the serologic study were mf positive (one in each category). Prevalences of CIA positivity were considerably higher than mf prevalences in these children, as has also been seen in other studies.¹³ However, the approximate similarity in mf and CIA positivity prevalences in the two categories of children provides stronger evidence that a potential difference in antibody response observed between the two categories of children would be related to the mothers mf status rather than to the current infection status of the children.

The ELISA antibody measurements provided only limited indications of intercategory differences in immune responsiveness. In study 1, no significant differences were observed in mean ELISA OD values between the three cate-

gories of adults. Rather, these values appeared to be related to the mf status at the time of serum sampling, and they followed the commonly seen pattern of higher IgG4 and lower IgG1, IgG2, IgG3, and IgE levels in mf-positive than in mf-negative individuals.^{11,13,15} In study 2, however, the mean IgG4 level was significantly higher in children born to mf-positive mothers than in those born to mf-negative mothers, suggesting that prenatal sensitization might lead to increased IgG4 responsiveness when the children are exposed to infection later in life. A similar trend, although not statistically significant, was seen in a recent follow-up study in the Cook Islands.¹⁶ According to the previously proposed role of IgG4 in blocking of IgE-mediated immunity mechanisms in helminth infections,^{17,18} the observed high levels of IgG4 would result in an increased susceptibility to filarial infection in children born to infected mothers, thus being in agreement with the higher prevalence of mf positivity in children born to mf-positive mothers than in those born to mf-negative mothers.⁵ Generally, the mean ELISA OD values for IgG1, IgG2, IgG4, and IgE were lower in study 1 than in study 2, probably reflecting the higher mean age in the former than in the latter study population.^{11,13}

The most intriguing findings appeared from the studies on antibodies to the sheath of *W. bancrofti* mf. Preliminary studies indicated that IgG2 was the predominating antibody isotype in this type of response, as has also been reported by others;^{19,20} therefore, only IgG2 measurements were used in the present study. In this respect, it is interesting to note that IgG2 antibody responses are regulated by the Th1 subset of T helper cells, and that Th1-like responses have been particularly associated with putatively immune individuals (i.e., amicrofilaremic and antigen-free) from areas endemic for Bancroftian filariasis.²¹ No relationship was observed between IgG2 ELISA OD values and IgG2 IFAT status probably because of the more complex composition and heterologous identity of antigens used in the former than in the latter test. It is also possible that the carbohydrate nature of the sheath antigens reported by others¹⁹ limits the detectability of the anti-sheath antibodies in ELISA.

It has been observed in several animal models of filariasis that appearance of antibodies to the mf sheath coincides with disappearance of mf from the circulation, i.e., with termination of the patent microfilaremic period of infection.²²⁻²⁵ Apparently the anti-sheath antibodies are involved in a mechanism eliminating mf in the infected hosts, probably through mediation of leukocyte adherence to and killing of mf, as has been observed *in vitro*.²⁴⁻²⁷ Anti-sheath antibodies have also been identified in sera from a proportion of humans from areas endemic for lymphatic filariasis.^{8,9,28,29} It was striking in these studies, as in the present study, that individuals who were positive for anti-sheath antibodies were also negative for mf, thus suggesting the involvement of these antibodies in an mf elimination mechanism in human filariasis as well. As opposed to the case for the animal models, however, the previous human studies could not place a time scale on the appearance and disappearance of these antibodies in relation to the microfilaremic patent period of infection.

By extrapolating from the animal model studies it would be expected in study 1 to find IFAT positivity predominantly among individuals who had changed from an mf-positive to

an mf-negative status (i.e., in category 2). Surprisingly, however, there were no IFAT-positive cases in this category. All were in category 3, namely among individuals who were mf negative at both examinations over the 16-year observation period. Not even the two persons in category 2 who had changed from mf positive to being both mf and CIA negative were IFAT positive. It cannot be excluded that other individuals had been IFAT positive some time in the period between 1975 and 1992, but the difference between categories 2 and 3 is so striking that a more fundamental difference appears to exist between the developmental sequence of these antibodies in the commonly used animal models and in human filariasis. In study 2, IFAT positivity occurred with a very high but almost similar prevalence in children born to both mf-positive and mf-negative mothers, thus suggesting that the IFAT status was not related to the mothers' mf status around the time of pregnancy.

When comparing studies 1 and 2, which were performed on individuals from the same endemic communities, it was obvious that the overall prevalence of IFAT positivity was considerably higher in the younger individuals of study 2 (mean age = 14 years; IFAT prevalence = 45.5%) than in the older individuals of study 1 (mean age = 48 years; IFAT prevalence = 16.7%). This is contradictory to what should be expected from predictions from the mentioned animal model studies. Rather, the findings present similarities to what was recently observed in infections with *Loa loa*, another human filaria. Antibodies to the sheath of *L. loa* are commonly seen in amicrofilaremic humans from areas with endemic loiasis.³⁰ When the time course of infection was followed in a mandrill/*L. loa* model, antibodies to the mf sheath were detected in the prepatent period only, and subsequently disappeared shortly before the onset of patency.³¹ A similar sequence in human Bancroftian filariasis could explain the high child to adult ratio in prevalence of IFAT positivity, as well as the occurrence of IFAT positivity only in category 3 of the adult study population seen in the present study. In addition to being mf negative, the majority of the IFAT-positive individuals (88% for study 1 and 2 combined) were also CIA negative. Therefore, IFAT positivity was highly correlated not only with mf negativity but also with CIA negativity, and it is tempting to speculate that IFAT-positive individuals represent a group of humans with an operating active resistance mechanism towards filarial infection. The combined observations from the present study thus suggest that antibodies to the sheath of mf are involved in an active resistance mechanism in human lymphatic filarial infection, which operates most vigorously in young individuals in the population in an endemic area and which breaks down in some individuals as they grow older (perhaps due to prolonged exposure), resulting in development of patent microfilaremia. Further studies are in progress to evaluate this hypothesis.

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