

## FILARIAL-SPECIFIC ANTIBODY RESPONSE IN EAST AFRICAN BANCROFTIAN FILARIASIS: EFFECTS OF HOST INFECTION, CLINICAL DISEASE, AND FILARIAL ENDEMICITY

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**Abstract.** The effect of host infection, chronic clinical disease, and transmission intensity on the patterns of specific antibody responses in Bancroftian filariasis was assessed by analyzing specific IgG1, IgG2, IgG3, IgG4, and IgE profiles among adults from two communities with high and low *Wuchereria bancrofti* endemicity. In the high endemicity community, intensities of the measured antibodies were significantly associated with infection status. IgG1, IgG2, and IgE were negatively associated with microfilaria (MF) status, IgG3 was negatively associated with circulating filarial antigen (CFA) status, and IgG4 was positively associated with CFA status. None of the associations were significantly influenced by chronic lymphatic disease status. In contrast, IgG1, IgG2, and IgG4 responses were less vigorous in the low endemicity community and, except for IgG4, did not show any significant associations with MF or CFA status. The IgG3 responses were considerably more vigorous in the low endemicity community than in the high endemicity one. Only IgG4 responses exhibited a rather similar pattern in the two communities, being significantly positively associated with CFA status in both communities. The IgG4:IgE ratios were higher in infection-positive individuals than in infection-negative ones, and higher in the high endemicity community than in the low endemicity one. Overall, these results indicate that specific antibody responses in Bancroftian filariasis are more related to infection status than to chronic lymphatic disease status. They also suggest that community transmission intensity play a dominant but subtle role in shaping the observed response patterns.

### INTRODUCTION

The contribution of parasite specific antibody responses to infection and chronic disease manifestations in lymphatic filariasis still remains unclear. Early work in this area that focused on antibody responses in microfilaremic individuals in comparison with individuals either with chronic lymphatic pathology or presumably exposed but displaying no outward signs of infection (so-called endemic normal individuals) suggested a differential expression of filarial-specific antibody responses in these patient categories.<sup>1–3</sup> In particular, these early studies suggested a dichotomy in the expression of specific antibody responses between the categories, namely a high specific IgG4 response and IgG4:IgE ratio in microfilaremic individuals compared with a more prominent IgG1, IgG2, and IgG3 response in patients with elephantiasis and endemic normal individuals. These findings have been central to immunologic explanations for the pathogenesis of chronic filarial disease: that microfilaremic individuals are largely hypo-responsive to filarial antigens as a result of carrying active infection while chronic pathology is the result of a subsequent gain of immune responsiveness to these antigens.

A major problem with these investigations, however, has been the inability to accurately diagnose the infection status of individuals by using detection of microfilariae in blood samples. The biases introduced by inaccurate infection classification in retarding a better understanding of the immunobiology of filariasis have been highlighted by Freedman.<sup>4</sup> Recent studies in which patient classification was improved considerably using parasite antigen detection have shown that

both cytokine responses, T cell proliferation, and specific humoral responses to filarial antigens may be more related to infection than to overt clinical manifestations.<sup>4,5</sup> These findings cast doubt on an immunologic etiology as a primary cause of chronic disease pathogenesis in filariasis and point to the critical need for accurate classification of infection status in any study attempting to determine the role of immune responses in the natural history of filarial infection and disease.

More recent studies have also highlighted the role that filarial endemicity or community transmission intensity may play in immune processes in filariasis.<sup>6–11</sup> Not only may specific anti-filarial antibody responses be related to transmission intensity, but acquired immunity and immunopathologic responses may also be functions of filariasis endemicity or level of parasite transmission.<sup>10–13</sup> Population dynamic studies of the stimulation and regulation of immune responses to parasitic infection have furthermore highlighted the key role that nonlinear interactions of immune components with exposure intensity may play in regulating either a host protective response or immunologic unresponsiveness (tolerance) to parasitic infection.<sup>14–17</sup> These findings suggest that in addition to infection or clinical status, anti-filarial immune responses in individuals are also related to the transmission intensity to which they are exposed in the community, a factor that thus clearly needs to be considered when investigating any association between an immune component and clinical states of filariasis in individuals.

The work presented here is part of a broader study of the immunoepidemiology of *Wuchereria bancrofti* infection in coastal East Africa.<sup>11,18</sup> In this report, we describe and evaluate the filarial-specific antibody responses from adults in two endemic communities differing in endemicity according to both parasitologic and clinical status in an attempt to define

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the role of such responses to parasite epidemiology, as well as to obtain further insights into the likely impact of endemicity on the observed immune response patterns in endemic populations.

## MATERIALS AND METHODS

**Study populations and study design.** The study was conducted 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. After informed oral consent to participate (from parents or guardians for those less than 15 years of age), individuals  $\geq 1$  year of age were examined clinically (for filariasis-related chronic manifestations) and parasitologically (for microfilariae [MF]), and a venous blood sample was collected for detection of circulating filarial antigen (CFA) and filarial-specific antibodies.

A description of the study communities has been reported elsewhere together with a presentation of findings from the clinical, MF, and CFA examinations.<sup>18</sup> Briefly, Masaika and Kingwede had 950 and 1,013 inhabitants  $\geq 1$  year of age, respectively. In Masaika, overall MF and CFA prevalences were 24.9% and 52.2%, respectively, 4.0% of adults ( $\geq 20$  years of age) had 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 had limb elephantiasis, and 5.3% of adult males had hydrocele. Thus, the endemicity of lymphatic filariasis was higher in Masaika than in Kingwede, a fact also reflected to a much higher level of transmission in the first community than in the second one (annual transmission potentials of 92.9 and 6.4, respectively, during the year preceding the surveys reported here).<sup>19</sup> The present report analyzes filarial-specific antibody (IgG1, IgG2, IgG3, IgG4, and IgE) responses in relation to host infection (MF and CFA), chronic clinical disease, and community filarial endemicity level. The study was reviewed and 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.

**Clinical and parasitologic examination.** Clinical examination of study individuals was carried out by an experienced clinician. Filariasis-related chronic manifestations were graded,<sup>18</sup> but grades have been omitted in this report. Instead, chronic manifestations of hydrocele  $\geq$  grade II ( $\geq 6.0$  cm) and lymphedema/elephantiasis  $\geq$  grade I (loss of contour, pitting edema) are referred to as hydrocele and elephantiasis, respectively.

Blood sampling for parasitologic examination started at 9:00 PM due to the nocturnal MF periodicity in the study area. From each individual, 100  $\mu$ L of blood obtained by finger prick was collected in a heparinized capillary tube and transferred to a tube with 1 mL of 3% acetic acid. Specimens were later examined in a counting chamber under a microscope, and the number of MF/mL were recorded, as previously described.<sup>18</sup>

**Preparation of serum.** Immediately after finger prick blood sampling, 5 mL of venous blood was collected in plain Vacutainer® (Becton Dickinson, Franklin Lakes, NJ). Serum was

separated by centrifugation after overnight clotting in a refrigerator, and sodium azide was added to a concentration of 15 mM as a preservative. Serum was initially frozen at  $-20^{\circ}\text{C}$  in the field, and later stored at  $-80^{\circ}\text{C}$  in the main laboratory until use. Before further handling and testing of sera, 3  $\mu$ L/mL of tri-N-butyl phosphate (T-4908; Sigma, St. Louis, MO) and 10  $\mu$ L/mL of Tween 80 (P-1754; Sigma) were added for elimination of lipid-coated virus.<sup>20</sup>

**Circulating filarial antigen.** Serum specimens were examined for CFA by using the TropBio enzyme-linked immunosorbent assay (ELISA) kit for serum specimens (catalog no. 03-010-01; TropBio Ltd. Pty., Townsville, Queensland, Australia). The test was performed according to procedures of the manufacturer and as previously described.<sup>21</sup> Serum specimens with an optical density (OD) value  $\geq$  standard 2 of the manufacturer ( $\geq 32$  antigen units) were considered positive for CFA, and specimens with an OD value  $\geq$  standard 7 of the manufacturer were assigned a fixed value of 32,000 CFA units.

**Measurement of filarial-specific antibodies.** Sera were examined for filarial-specific IgG1, IgG2, IgG3, IgG4, and IgE antibodies by an ELISA. Antigen was prepared from *Brugia pahangi* adult worms recovered from experimentally infected jirds (*Meriones unguiculatus*). Worms were washed in phosphate-buffered saline, pH 7.4, containing 17.4 mg of protease inhibitor (phenylmethylsulfonyl fluoride), 50 mg of enzyme inactivator (L-1-tosylamide-phenylchloromethyl-ketone), and 2.5 mg of papain and trypsin inhibitor (N-a-p-tosyl-L-lysine-chloro-methyl-ketone hydrochloride) per 100 mL, and sonicated in an ice bath at maximum amplitude for 5 minutes (15-second sonication bursts and 30-second rest intervals). The homogenate was incubated overnight at  $4^{\circ}\text{C}$ . It was then centrifuged at 11,000 rpm for 20 minutes, and the supernatant was filtered through a 0.45- $\mu$ m filter (Minisart RC 15; Sartorius, Goettingen, Germany). The protein concentration measured with the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) was 2.5 mg/mL. The antigen was kept at  $-80^{\circ}\text{C}$  until use.

Optimal dilutions of antigen, serum, and conjugate were determined by titration. Buffers were prepared according to the procedures of Voller and Savigny.<sup>22</sup> Prior to measurement of IgE, sera were absorbed with a protein A agarose bead suspension (Ken-En-Tec A/S, Copenhagen, Denmark) at a ratio of 50:140 to remove blocking IgG4 antibodies.<sup>23</sup> Microtiter plates (Immuno-plates, Maxisorp 442404; Nunc A/S, Roskilde, Denmark) were coated by overnight incubation at  $4^{\circ}\text{C}$  with 100  $\mu$ L/well of *B. pahangi* antigen (diluted in coating buffer [0.03 M  $\text{Na}_2\text{CO}_3$ , 0.07 M  $\text{NaHCO}_3$ , pH 9.6] to give a protein concentration of 1  $\mu$ g/mL for IgG1, IgG2, IgG3, and IgG4, and 2  $\mu$ g/mL for IgE). Subsequent steps were carried out at room temperature. After three washes (three minutes/wash) with washing buffer (0.3 M NaCl, 0.003 M  $\text{KH}_2\text{PO}_4$ , 0.015 M  $\text{Na}_2\text{HPO}_4$ , 0.006 M KCl, 0.05% Tween 20, pH 7.4), 200  $\mu$ L of 0.5% bovine serum albumin in washing buffer was added to each well as a blocking agent and incubated for one hour. Plates were then washed as above, and incubated with 100  $\mu$ L of test serum diluted in washing buffer (incubation for 1.5 hours with 1:1,500, 1:500, 1:250, and 1:1,000 serum dilutions for IgG1, IgG2, IgG3, and IgG4, respectively, and incubation overnight with a 1:20 protein A-absorbed serum dilution for IgE). Plates were washed as above, and incubated with 100  $\mu$ L of horseradish peroxidase

(HRP)-conjugated antisera diluted in washing buffer (incubation for one hour with 1:1,500, 1:500, 1:500, and 1:2,000 dilutions of HRP-conjugated mouse anti-human monoclonal antibodies to IgG1, IgG2, IgG3 and IgG4 (CLB, Amsterdam, The Netherlands) respectively, and incubation for two hours with a 1:1,000 dilution of polyclonal rabbit-anti-human IgE (Dakopatts A/S, Glostrup, Denmark). After washing as before, 100  $\mu$ L of *o*-phenylenediamine (OPD) substrate solution prepared from OPD tablets (Dakopatts A/S) according to the manufacturer's instructions was added to each well. The reaction was stopped after reasonable development of color (maximum = 20 minutes) by adding 50  $\mu$ L of 2.5 M H<sub>2</sub>SO<sub>4</sub> per well. The OD values were measured at 492 nm using an ELISA reader (Bio-Rad Laboratories). Serum samples were tested in triplicate and the mean OD value was calculated. The OD value of a positive control serum included on all plates was used to adjust for minor plate-to-plate variations.

**Data analysis.** The primary analyses are all based on parasitologic and antibody intensity data measured in individuals grouped into various infection and clinical categories. Geometric mean intensities (GMIs) of microfilaremia, antigenemia, and filarial-specific antibody levels were calculated as  $\text{antilog}[(\sum \log x + 1)/n] - 1$ , with  $x$  the number of MF/mL, number of CFA units, and ELISA OD values, respectively, and  $n$  the number of individuals included. The IgG4:IgE ratios were first calculated for individual sera, and IgG4:IgE ratio GMIs were thereafter calculated as described above.

Two-group comparisons were carried out using either a simple chi-square test (for prevalence data) or a *t*-test (for continuous data). Antibody intensities between the two asymptomatic groups and individuals with chronic disease were analyzed with generalized linear models (GLMs) using Gaussian errors, with antibody intensity (log transformed OD values) as response and clinical and infection status as factors. One-factor tests based on F-values from the Gaussian GLM were used to compare each the GMIs of each antibody in relation to infection or disease status. Differences in antibody GMIs between communities were analyzed by two-factor GLMs with antibody OD values as responses and infection status and community as factors. *Post hoc* pairwise comparisons of groups to evaluate differences between each individual group were carried out using the Tukey method. A *P* value < 0.05 was considered statistically significant for all tests.

## RESULTS

**Characteristics of the study populations.** A total of 817 and 763 individuals in Masaika and Kingwede, respectively, were clinically examined and tested for MF, CFA and filarial-specific IgG1, IgG2, IgG3, IgG4, and IgE antibodies. Since chronic lymphatic disease manifestations in both communities were confined to individuals  $\geq 20$  years of age, only this age group was included in the present analyses (the effect of age on antibody responses will be analyzed in a separate report). Thus, the study populations were 440 individuals from Masaika and 312 individuals from Kingwede (87.0% and 71.4% of eligible individuals  $\geq 20$  years of age in these communities, respectively).

Table 1 shows the two study populations grouped according to MF and CFA status. In Masaika, the three infection status groups (MF and CFA negative, MF negative but CFA posi-

TABLE 1  
Characteristics of the study populations in Masaika and Kingwede  $\geq 20$  years of age, according to their infection status\*

	Infection status		
	MF-/CFA-	MF-/CFA+	MF+/CFA+
<b>Masaika</b>			
No. examined	151	135	154
Males/females	66/85	50/85	97/57
Mean age in years (range)	38.1 (20–84)	39.8 (20–80)	41.6 (20–80)
MF GMI, MF/mL	–	–	482
CFA GMI, units	7	2,667	20,941
<b>Kingwede</b>			
No. examined	233	61	18
Males/females	77/156	25/36	12/6
Mean age in years (range)	36.4 (20–76)	39.5 (20–76)	38.0 (20–78)
MF GMI, MF/mL	–	–	146
CFA GMI, units	8	344	13709

\* MF = microfilaria; CFA = circulating filarial antigen; GMI = geometric mean intensity (based on all examined individuals).

tive, and MF and CFA positive) contained an approximately equal proportion of the individuals. In Kingwede, the first of these groups was much larger than the second group, which was again much larger than the third group. There was no statistically significant difference in mean age between groups in any of the communities. In both Masaika and Kingwede, individuals positive for both MF and CFA had a significantly higher male-to-female ratio than those in the other two groups ( $\chi^2 = 21.4$ , degrees of freedom [df] = 2,  $P < 0.001$  and  $\chi^2 = 8.8$ , df = 2,  $P = 0.012$ , respectively). In both communities, the CFA GMI was significantly higher among the MF+ CFA+ individuals than among the MF- CFA+ individuals ( $t = -10.6$ , df = 162.2,  $P < 0.001$  and  $t = -9.6$ , df = 77,  $P < 0.001$ , for each community).

Table 2 shows the study populations grouped according to chronic disease and infection status. Elephantiasis was confined to the legs. One male in Masaika who had both hydrocele and elephantiasis was assigned to the elephantiasis group. The mean age was significantly higher in symptomatic individuals than in asymptomatic individuals in both Masaika (48.0 versus 38.3 years;  $t = 4.6$ , df = 93.6,  $P < 0.001$ ) and Kingwede (53.8 versus 36.6 years;  $t = 3.4$ , df = 310,  $P = 0.001$ ). Thirty-four (63.0%) of 54 males with hydrocele and 7 (63.6%) of 11 individuals with elephantiasis in Masaika were positive for CFA.

**Antibody responses in relation to infection status in asymptomatic individuals in Masaika.** Figure 1 shows the intensity of specific IgG1, IgG2, IgG3, IgG4, and IgE responses in relation to infection status among asymptomatic individuals in Masaika (left column). Results show that intensities of specific IgG1, IgG2, IgG3, and IgE were highest among individuals negative for both MF and CFA, and lowest in individuals positive for both MF and CFA. The opposite pattern was seen for specific IgG4 antibody. Here, intensities were highest among individuals positive for both MF and CFA and lowest among individuals negative for both MF and CFA. Similar test patterns were observed for prevalence.

Pairwise infection group comparisons carried out on the observed specific antibody intensities among the asymptomatic individuals in Masaika are shown in Figure 2 (left column). IgG1, IgG2, IgG3, and IgE showed a rather similar

TABLE 2

Characteristics of the study population in Masaika and Kingwede  $\geq 20$  years of age, according to clinical presentation and infection status\*

Clinical category	No. examined	Mean age in years (range)	Infection status		
			MF-/CFA-	MF-/CFA+	MF+/CFA+
<b>Masaika</b>					
Asymptomatic	368	38.3 (20-80)	120	122	126
Hydrocele	54	48.7 (23-76)	20	10	24
Elephantiasis	18	45.8 (20-84)	11	3	4
<b>Kingwede</b>					
Asymptomatic	304	36.6 (20-76)	227	61	16
Hydrocele	5	60.2 (44-78)	3	0	2
Elephantiasis	3	43.0 (30-67)	3	0	0

\* For definitions of abbreviations, see Table 1.

pattern of progressive decrease from the uninfected (group 1) to the MF- CFA+ (group 2) and MF+ CFA+ (group 3) individuals. For IgG1, IgG2 and IgE, the decrease in intensity levels between uninfected and MF- CFA+ groups was not significant, but that between uninfected and MF+ CFA+ groups was significant (i.e., when group 3 is compared with group 1 in Figure 2). This extra effect of being MF positive was also indicated by the fact that there was an additional significant decrease in antibody intensity when comparing the MF- CFA+ and MF+ CFA+ groups directly (i.e., group 2 versus group 3) for IgG1 and IgG2. For IgG3, the decrease in antibody response was significant even when individuals became positive for CFA only, with the decrease becoming more marked (although not significantly) in those individuals with both MF and CFA. The results clearly imply that being MF positive in addition to being CFA positive can have an added suppressive effect on the production of IgG1, IgG2, IgG3, and possibly IgE in patients with filariasis. In contrast, IgG4 was positively associated with CFA status, irrespective of MF status (no significant difference between groups 2 and 3 in Figure 2).

**Antibody responses in relation to infection status in symptomatic individuals in Masaika.** Table 3 compares the age, infection characteristics, and specific antibody responses of males with hydrocele and individuals with elephantiasis in Masaika. There were no significant differences in these parameters between the two groups. The groups were therefore combined to form a single group of individuals with chronic symptomatic disease for further analyses.

The intensity of specific IgG1, IgG2, IgG3, IgG4, and IgE responses in relation to infection status among symptomatic individuals in Masaika is shown in Figure 1 (right column). It showed a remarkably similar pattern to that observed for asymptomatic patients (although only significant for IgG1 and IgG3, most likely due to smaller sample sizes). Furthermore, pairwise inter-group analysis of the associations between antibody intensities and MF and CFA status gave comparable results to those seen for the asymptomatic individuals in Figure 2.

**Effect of interaction between infection and clinical status on antibody responses in Masaika.** The effects of infection (MF- CFA-, MF- CFA+, or MF+ CFA+) and clinical (asymptomatic versus symptomatic) status on specific antibody intensities were evaluated for the entire study population in Masaika by the fit of a two-factor Gaussian GLM to the antibody mean intensity data (Table 4). The results show that the association between antibody intensity and infection status was highly significant for all antibodies. IgG4 intensity

was also associated with clinical status, although less significantly, being higher among symptomatic individuals than among asymptomatic individuals. The IgG1 and IgG2 intensities were marginally associated with clinical status, with these antibodies also being higher among symptomatic individuals than among asymptomatic individuals. Most importantly, however, the present results indicate that clinical status did not significantly influence the association between the intensity of the measured specific antibodies and infection status (the interaction term in the GLM model not significant in each case).

**Effect of filarial endemicity on antibody responses among asymptomatic individuals in Masaika and Kingwede.** Only a few individuals in Kingwede had chronic disease (Table 2), and the effect of filarial endemicity on specific antibody profiles was therefore examined by comparing the antibody intensity patterns among asymptomatic individuals in Masaika and Kingwede in relation to infection status (Figure 3). Overall, intensities of IgG1, IgG2, and IgG4 were higher in Masaika than in Kingwede, whereas surprisingly the opposite was seen for IgG3. The obvious decrease in intensity of IgG1, IgG2, IgG3, and IgE seen in Masaika when moving from MF- CFA- individuals to MF+ CFA+ individuals was not seen in Kingwede (Figure 3; compare the inter-group results shown in Figure 2 for Kingwede versus Masaika). However, Kingwede still showed a clear increase in intensity of IgG4, when moving in this direction, with this increase showing a significant positive association with CFA status (Figure 2).

**IgG4:IgE ratios in relation to infection status, clinical status, and filarial endemicity.** The IgG4:IgE intensity ratios in relation to infection (MF- CFA-, MF- CFA+, and MF+ CFA+) and clinical (asymptomatic versus symptomatic) status in Masaika are shown in Figure 4a. In both clinical groups, the ratios were lowest among MF- CFA- individuals and highest among MF+ CFA+ individuals. For each infection status group, the ratios were higher among symptomatic than among asymptomatic individuals, but this was only statistically significant for individuals negative for both MF and CFA ( $t = 3.199$ ,  $df = 39.61$ ,  $P = 0.002$ ).

The IgG4:IgE ratios in relation to infection status among asymptomatic individuals are compared between Masaika and Kingwede in Figure 4b. In both communities, the ratios were lowest among the MF- CFA- group and highest among the MF+ CFA+ group. For each infection status group, the ratios were significantly higher in Masaika than in Kingwede (Gaussian GLM model: village  $\times$  infection status term:  $F = 12.27$ ,  $df = 1,676$ ,  $P < 0.0001$ ).



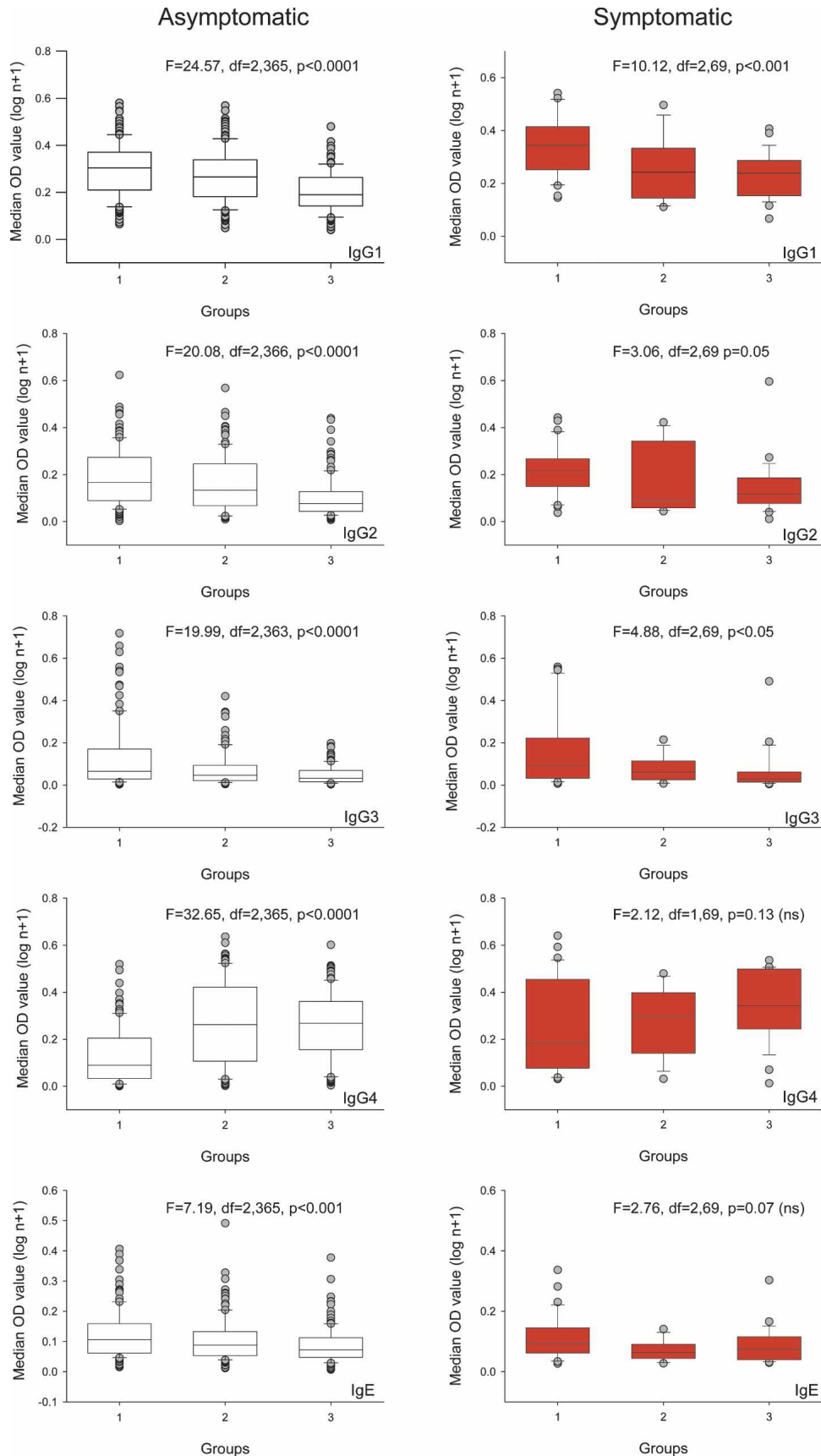


FIGURE 1. Intensity of specific IgG1, IgG2, IgG3, IgG4, and IgE responses (log optical density [OD] values) in relation to infection status among asymptomatic individuals (left column, open bars) and individuals with chronic filarial disease (right column, closed bars) in Masaika. Groups 1-3 represent MF-/CFA-, MF-/CFA+, and MF+/CFA+, respectively. Box-shaped bars show median (middle line across the bar), interquartile range (vertical ends of the bars), outliers (o), and whiskers (lines extending from the bar to the highest and lowest values excluding the outliers). Results from a one-factor GLM testing for the effect of infection status on antibody response is given for each graph. MF = microfilaria; CFA = circulating filarial antigen; df = degrees of freedom. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

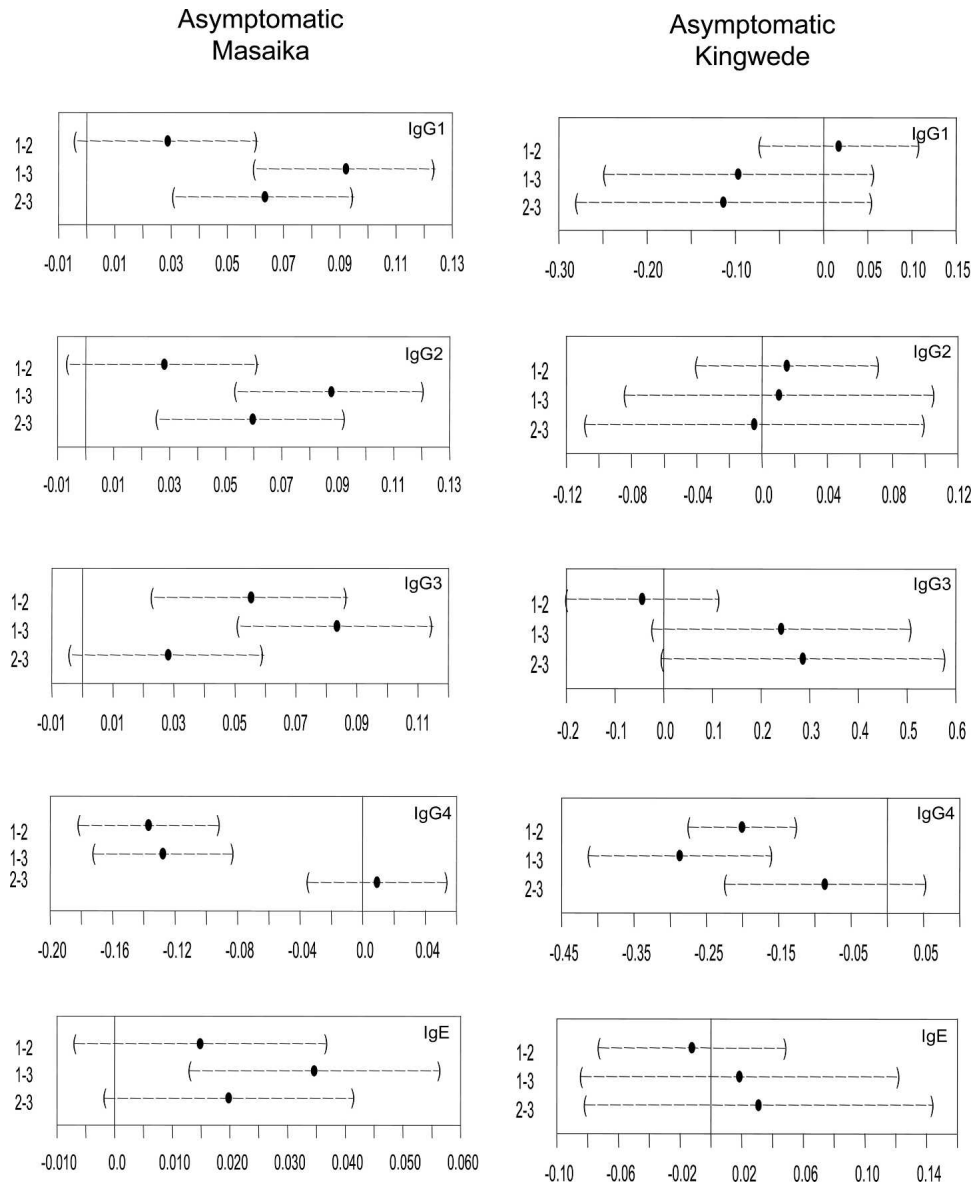


FIGURE 2. Standard error of the difference ( $\pm$  95% confidence interval) in mean antibody intensity in relation to infection status among asymptomatic individuals in Masaika (left column) and Kingwele (right column), for filarial-specific IgG1, IgG2, IgG3, IgG4, and IgE. Groups 1-3 represent MF-/CFA-, MF-/CFA+, and MF+/CFA+, respectively. On the y-axes, 1-2 indicates group 1 versus group 2, etc. Black dots indicate estimated standard errors of differences in mean antibody intensities between two respective infection groups. Broken lines indicate the 95% confidence intervals of the specified standard errors. In this Tukey multiple group comparison method, where the lines do not reach the zero mark, this would indicate a significant difference in mean antibody intensities between two infection groups. Note that a positive difference (dots and broken lines to the right of the zero line) would indicate a higher mean response in the first group in each two-group comparison, and a negative difference (dots and broken lines to the left of the zero line) would indicate the opposite, i.e., a lower mean response in the first comparator group. MF = microfilaria, CFA = circulating filarial antigen.

DISCUSSION

Numerous immunologic studies have been carried out in lymphatic filariasis in which the presence of MF has been used as an indicator of infection. Newly developed diagnostic techniques, such as detection of CFA and use of ultrasound, have shown that many more individuals in disease-endemic areas are infected with adult worms than those identified with MF.<sup>24,25</sup> Since active infection may profoundly affect immune responses,<sup>26</sup> it is imperative that for immunologic studies into the association of immune components with parasitic infection and disease processes, individuals be classified accurately

both in terms of clinical and infection status.<sup>4,27</sup> The present study thus analyzed specific antibody responses to *W. bancrofti* in disease-endemic populations of clinically and parasitologically well-characterized individuals. The infection classification scheme we followed also facilitated a more refined characterization of the sequel of filarial infection in an endemic community by allowing us to divide individuals into three infection groups based on MF and CFA detection. The first of these was negative for both MF and CFA, and thus can be considered infection free. Since the study individuals were adults who had grown up in communities with active transmission, it is likely that most of those in group one had been

TABLE 3

Characteristics, infection status, and filarial-specific antibody responses of individuals with chronic disease manifestations in Masaika\*

	Hydrocele	Elephantiasis	Statistics†
No. individuals (Males/females)	54 (-)	18 (6/12)	-
Mean age, years	48.7	45.8	t = 0.65, P = 0.52
No. MF positive (%)	24 (44.4)	4 (22.2)	$\chi^2 = 2.81, P = 0.094$
No. CFA positive (%)	34 (63.0)	7 (38.9)	$\chi^2 = 3.19, P = 0.074$
MF GMI* (MF/mL)	9.8	2.2	t = 1.56, P = 0.12
CFA GMI* (units)	730	114	t = 1.77, P = 0.081
No. positive for antibody (%)			
IgG1	51 (94.4)	17 (94.4)	$\chi^2 = 0.000, P = 1.00$
IgG2	32 (59.3)	10 (55.6)	$\chi^2 = 0.076, P = 0.78$
IgG3	14 (25.9)	4 (22.2)	$\chi^2 = 0.099, P = 0.75$
IgG4	54 (100)	18 (100)	-
IgE	38 (70.4)	13 (72.2)	$\chi^2 = 0.022, P = 0.88$
Mean antibody intensity‡ (optical density)			
IgG1	0.912	0.953	t = -0.29, P = 0.77
IgG2	0.515	0.520	t = -0.043, P = 0.97
IgG3	0.285	0.266	t = 0.17, P = 0.87
IgG4	0.989	0.805	t = 0.90, P = 0.37
IgE	0.241	0.246	t = -0.094, P = 0.93

\* For definitions of abbreviations, see Table 1.

† 70 degrees of freedom for all t-tests.

‡ Geometric mean intensity (based on all examined individuals).

exposed and perhaps had developed some type of resistance to *W. bancrofti* infection. The second was negative for MF but positive for CFA. Thus, despite the presence of adult worms, no MF were detected. This was probably due to the MF being too few for detection by microscopy, immune mechanisms that destroyed produced MF, or adult single-sex infections. The third group was positive for both MF and CFA and therefore harbored actively MF producing adult worms. A significantly higher CFA intensity among individuals in this group

TABLE 4

Analysis table for generalized linear model (GLM) fits to antibody intensity data\*

	Residual deviance	F	df	Probability (F)
IgG1				
Infection status	4.908	65.627	1,438	0.000
Clinical status	4.870	3.414	1,437	0.065
Infection: clinical status	4.863	0.677	1,436	0.411
IgG2				
Infection status	5.477	45.113	1,438	0.000
Clinical status	5.430	3.771	1,437	0.053
Infection: clinical status	5.427	0.205	1,436	0.651
IgG3				
Infection status	5.274	48.261	1,436	0.000
Clinical status	5.250	2.067	1,435	0.151
Infection: clinical status	5.244	0.418	1,434	0.518
IgG4				
Infection status	10.667	44.705	1,438	0.000
Clinical status	10.349	13.438	1,437	0.002
Infection: clinical status	10.333	0.673	1,436	0.413
IgE				
Infection status	2.167	16.731	1,438	0.000
Clinical status	2.158	1.725	1,437	0.189
Infection: clinical status	2.157	0.132	1,436	0.716

\* GLM fit to the log transformed optical density (OD) values using Gaussian errors with antibody intensity (log transformed OD values) as response and infection status and clinical status as factors. Clinical status = asymptomatic or chronic disease (hydrocele or/and elephantiasis). Infection status = MF-CFA-, MF-CFA+, or MF+CFA+. A P value < 0.05 in respect to clinical status and for infection status indicates significant association between antibody intensity and clinical status or between antibody intensity and infection status as the case may be. A P value < 0.05 for clinical status × infection status indicates a significant influence of clinical status on the association between the intensity of the measured antibody and infection status. df = degrees of freedom. For definitions of other abbreviations, see Table 1.

compared with the second group in both our study communities suggests that the former had higher adult worm loads than the latter. Apart from increasing the sensitivity of detecting infection in individuals, any observed association and patterns of change of antibody responses with this natural ordering of infection states is thus also expected to provide a stronger indication of the role of immunity in the filarial infection process.

To investigate the impact of disease status, we further categorized individuals after clinical examination as being either asymptomatic or having chronic lymphatic pathology (hydrocele and/or elephantiasis). Males with hydrocele and individuals with leg elephantiasis consisted of a mixture of MF- CFA- individuals, MF- CFA+ individuals, and MF+ CFA+ individuals. Those individuals with chronic disease were therefore heterogeneous with respect to infection status, as also observed by others.<sup>4,28-31</sup> Nesting such infection categories within the two clinical groups would therefore be important to account for the confounding impact of infection on any effects of filarial disease on observed immune responses. Note, however, that the number of individuals with chronic disease was large enough only in the community with high endemicity to allow for analysis. In this community, there was no significant age difference between males with hydrocele and individuals with elephantiasis. This finding and the fact that overall there was a similarity in antibody responses between these two groups further justified our merging of these individuals into one group of individuals with chronic disease for carrying out the analyses of the impact of disease reported.

A first major finding of this study is that when analyzed in relation to infection status, the clinical status of an individual did not significantly influence the observed association between the profiles of specific antibodies and infection stage. This may appear to contrast with findings of earlier studies, which suggested that individuals with chronic disease produce higher amounts of specific IgG1, IgG2, IgG3, and IgE, and lower amounts of IgG4 than asymptomatic individuals, which was assumed to reflect a role for immunity in the develop-

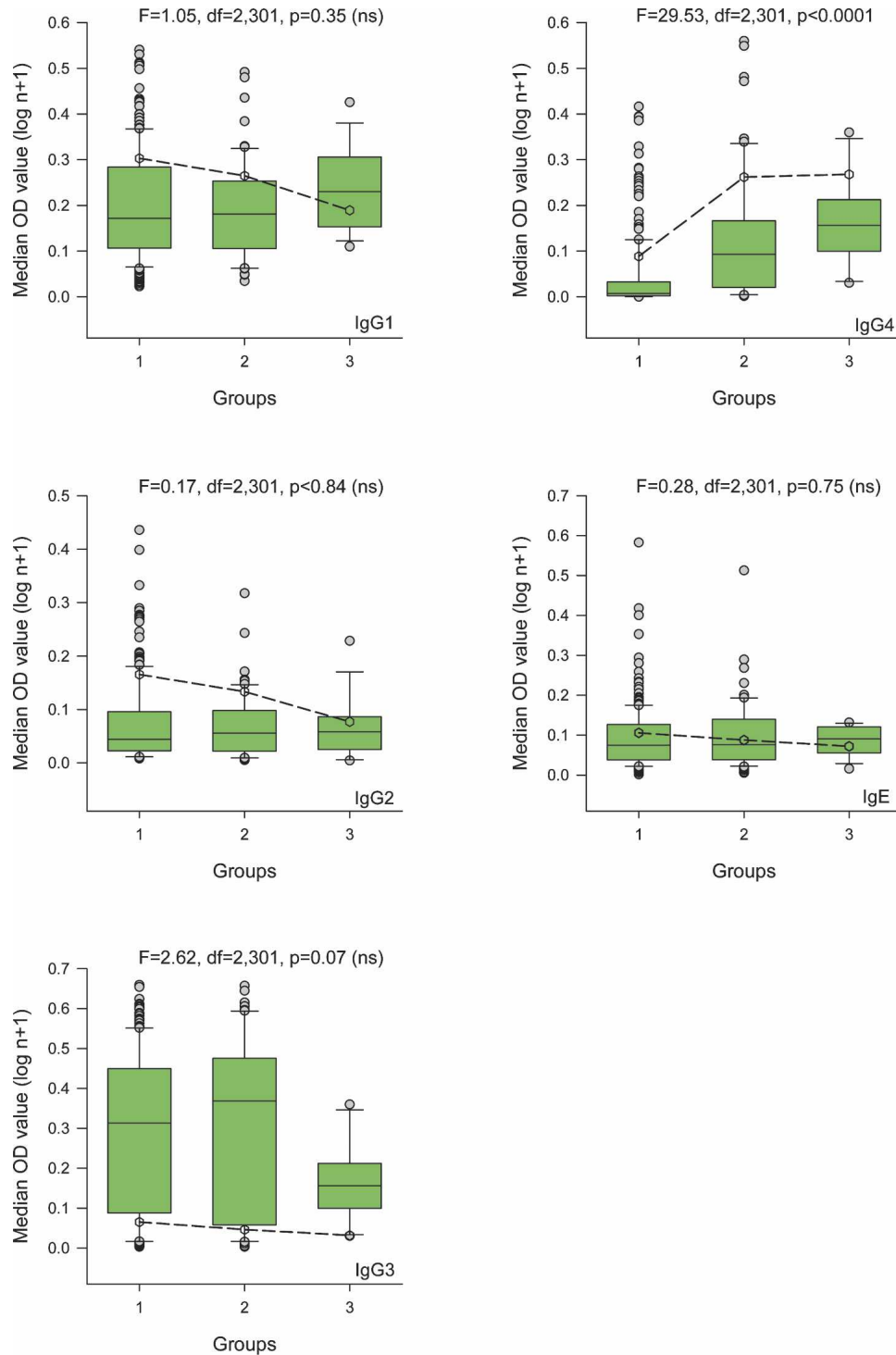


FIGURE 3. Intensity of specific IgG1, IgG2, IgG3, IgG4, and IgE responses in relation to infection status among asymptomatic individuals in Kingwede. Bars and groups are described in Figure 1. The dotted line in each graph shows the comparison median response in each infection group obtained among the asymptomatic individuals in Masaika. Results from a one-factor GLM testing for the effect of infection status on antibody response is given for each graph. OD = optical density; df = degrees of freedom; ns = not significant. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

ment of chronic filarial disease.<sup>1,26,32</sup> These results, in marked contrast to the present results, highlighted the very high intensities of specific IgE in diseased individuals, and suggested that specific IgE may be involved in development of the chronic lymphatic pathology. More recent studies, which have used more precise characterization of individuals in relation

to infection status, have, similar to the present study, suggested that antibody responses are more related to the presence or absence of MF and/or CFA than to disease.<sup>5,7,33,34</sup> In agreement with this, T cell proliferation and lymphocyte cytokine responses have also been shown to be primarily associated with the presence or absence of active infection rather



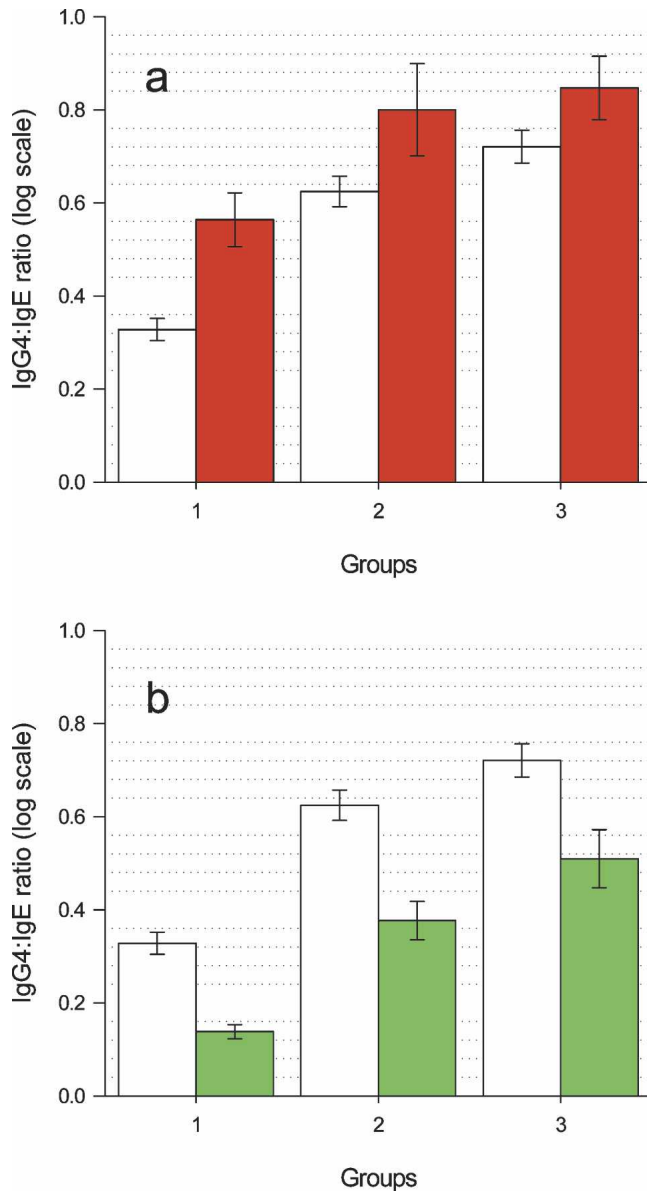


FIGURE 4. Mean specific IgG4:IgE ratios ( $\pm$  SE) in relation to infection status among **a**, asymptomatic individuals (open bars) and individuals with chronic disease (filled bars) in Masaika, and **b**, asymptomatic individuals in Masaika (open bars) and in Kingwede (filled bars). This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

than clinical status.<sup>27</sup> The poor relationship between immunologic responses and clinical disease is consistent with the more limited role presently attributed to human anti-parasite responses in the development of filarial lymphatic pathology.<sup>35,36</sup> Although the results of the present study could reflect an artifact of combining both hydrocele and lymphoedema patients into one diseased group (it has been suggested that hydrocele may be induced more by worm burden, and that lymphoedema could have a more complicated pathologic pathway<sup>35</sup>), it is notable that it supports more recent epidemiologic evidence that in areas of low-to-moderate transmission, worm burden effects, rather than immunity, may constitute the major risk factor for the development of lymphoedema.<sup>11</sup>

The finer definition of infection status afforded by our

study design has yielded further important findings regarding the association of filarial antibody responses with MF and adult worm infections. Thus, the highest intensities of specific IgG1, IgG2, IgG3, and IgE in the high endemicity community were among the MF- CFA- (i.e., uninfected) individuals, and the lowest were among CFA+ MF+ individuals (those harboring MF and adult worms). Studies in other disease-endemic areas have similarly found low levels of these responses associated with the presence of MF.<sup>5,7,28,31,33,34,37</sup> Thus, it would appear that specific IgG1, IgG2, IgG3, and IgE production is suppressed by both adult worm and MF infections or suppresses filarial infection. Closer inspection of our data show that this negative effect of infection appeared to be related to MF status. Although the intensity of specific IgG1, IgG2, and IgE responses were reduced in MF- CFA+ individuals, the reductions were significantly different from uninfected individuals only among MF+ CFA+ individuals. Our findings thus suggest either a further suppressive role for MF on the generation of the responses or that these antibodies may be suppressing MF intensity in CFA+ individuals. It is also possible that the apparent role of MF to some extent may be a reflection of the higher intensity of adult worms detected in the MF+ CFA+ individuals. Specific IgG3, was clearly negatively associated with CFA, a trend also observed by others.<sup>5,7,31</sup> Thus, it would appear that specific IgG3 production is suppressed by or suppresses the burden of adult worm infection, being minimally affected by the presence of MF. In contrast to the other antibodies, specific IgG4 showed a significant positive association with CFA. Specific IgG4 production thus appeared to be induced by the adult worms, or it enhanced their survival, e.g., by blocking effector immune responses that eliminate adult worms. Such positive association between specific IgG4 and adult worms has also been reported by others.<sup>5,7,31,34,37</sup>

The influence of transmission intensity on specific antibody patterns was examined by comparing responses in the high and low endemicity study communities. Such a community-based comparative study design has been proposed to be an important requirement for investigating the role of immunologic processes as determinants of observed infection and disease patterns because it essentially enables the addressing of exposure-related factors as confounders of any observed immunity-related association.<sup>16,38-41</sup> Due to reasons mentioned earlier, the present analysis was restricted to the adult asymptomatic individuals. Among these, specific IgG1, IgG2, and IgG4 responses were markedly higher in the high endemicity community than in the low endemicity one, while surprisingly the opposite was seen for IgG3. The reason for the significant inverse relationship between endemicity level and specific IgG3 response is not clear, but a similar observation has been made for another human filaria, *Loa loa*.<sup>42</sup> IgG3 is thought to have the ability to neutralize pathogens at the port of entry into the body via Fc $\gamma$ R-mediated effector responses.<sup>43</sup> The inverse relationship between high IgG3 levels and low infection burden in the low transmission community may therefore suggest a protective role for IgG3 against incoming adult worms in that community. The opposite situation observed in Masaika may suggest that as transmission intensity increases this protective effect is down-regulated by the increased worm burdens in the highly exposed community. Given that IgG3, like IgG1 and IgG2, is part of the Th1 immune response, which is believed to be implicated in chronic disease

development in lymphatic filariasis,<sup>10</sup> one reason for down-regulation could be that this antibody plays a more important role (than IgG1 and IgG2 for example) in disease development. This is supported by the fact that although the lymphoedema:infection ratio in Kingwede was approximately 0.12, it was significantly lower (0.08) in Masaika.

When antibody responses were analyzed in relation to infection status, only IgG4 showed a similar pattern in the high- and the low-endemicity communities (i.e., a significant positive association with CFA). Thus, using specific IgG4 as an immunologic marker of community infection burden<sup>26,44</sup> appears to be valid irrespective of endemicity level, although many more individuals are positive for specific IgG4 than for infection. In sharp contrast to findings in the high-endemicity community, specific IgG1, IgG2, IgG3, and IgE responses were not significantly related with infection (either CFA or MF) status in the low-endemicity community. Thus, the association of these antibody responses with infection status appears to change with transmission intensity, although clearly differences in infection intensity (suggesting threshold infection burdens?) may also be involved.

Our investigation of the specific IgG4 and IgE responses has also produced new findings regarding the potential effect of these antibodies in development of chronic disease. Specific IgG4 and IgE show a high degree of parallel antigen recognition in lymphatic filariasis, and IgG4 may act as a blocking antibody that protects the host from IgE-mediated hypersensitivity reactions.<sup>45</sup> A 10 times higher IgG4:IgE ratio among MF+ asymptomatic individuals than among individuals with chronic filarial disease led to the suggestion that specific IgE may be mediating pathogenic effects, but that sufficient IgG4 levels may be protective against such effects.<sup>32</sup> However, the same study also found the IgG4:IgE ratio was lowest among asymptomatic MF- individuals, thereby suggesting that high levels of IgE with low levels of IgG4 was not in itself sufficient to initiate pathologic effects.

In the present study, the IgG4:IgE ratio in the high-endemicity community was higher among individuals with chronic disease than among asymptomatic individuals in all infection groups, although this difference was only significant among those negative for both MF and CFA. Therefore, these results did not support general involvement of specific IgE in the development of chronic lymphatic pathologic effects. In schistosomiasis, a low IgG4:IgE ratio has been associated with acquired immunity.<sup>46</sup> A study of Brugian filariasis patients similarly suggested that the IgG4:IgE ratio can serve as an indicator of permissiveness or resistance to infection.<sup>32</sup> The observation in the present study of lowest IgG4:IgE ratios among individuals negative for both MF and CFA, and highest ratios among individuals positive for both MF and CFA in both communities (also reported by Nicolas and others<sup>5</sup>) is consistent with this theory. However, if resistance is exposure driven as has been suggested,<sup>11,13</sup> and a low IgG4:IgE ratio is used as an indicator of resistance, then a lower ratio would be expected in the high-endemicity community than in the low-endemicity community in general, and in particular among the asymptomatic individuals negative for both MF and CFA. The fact that this was not observed in the present study indicates that matters may be more complicated. This and the other findings of this study implicating the likely occurrence of subtle interactions between exposure intensity and infection states on specific antibody responses to

*W. bancrofti* also suggest that there is an urgent need to gain a better understanding of how the current global program to eliminate lymphatic filariasis will alter immune responses, and thus ultimately affect both infection dynamics and filarial pathogenesis in mass-treated disease-endemic communities.<sup>47,48</sup>

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