

Differential Expression of IgE and IgG4 Specific Antibody Responses in Asymptomatic and Chronic Human Filariasis¹

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ABSTRACT. A population of 164 adult individuals resident in an area endemic for *Brugia malayi* lymphatic filariasis has been studied for humoral immune responses to filarial parasites. Antibody levels to Ag extracted from adult worms were determined for each of the IgG subclasses, for IgM and for IgE. The dominant isotype of antifilarial antibody was IgG4, which represented 88% of total IgG in asymptomatic microfilareemics, most of whom possessed 100 to 1000 µg/ml of specific antibody of this subclass (geometric mean 762 µg/ml). Patients with chronic disease (elephantiasis), who were generally amicrofilareemic, had substantially higher levels of IgG1, IgG2, and IgG3, but a 3.4-fold lower geometric mean level of specific IgG4 (222 µg/ml) than asymptomatics with or without microfilaremia. In contrast, specific IgE antibody levels in cases of elephantiasis were on average 4.5 times higher than those found in the asymptomatic carrier state. The majority of microfilareemics were therefore typified by extremely high specific IgG4 concentrations and relatively low IgE reactivities, whereas clinical cases tended to show the reverse relationship. The possible roles of these isotypes and the implications of changing IgG4/IgE balances in disease are discussed. *Journal of Immunology*, 1993, 150: 3941.

The human immune response to filarial infections bears a number of striking features. First, like many helminths, filarial worms stimulate high levels of IgE (1–3) giving support to the hypothesis that this isotype evolved to combat extracellular metazoan parasites (4). Second, eosinophilia is a common sign of filarial infection (5), which can more rarely be manifest in a hyper-

eosinophilic condition (6). Third, and most recent, it has been recognized that the IgG4 subclass is unusually predominant among specific antifilarial antibodies, representing 50 to 95% of the total IgG response (7, 8) in contrast to a normal serum proportion of 4% (9).

The relationship between IgE and IgG4 is intriguing. In filariasis, there is a remarkable qualitative parallelism between these isotypes (10). One explanation for this observation may be that both depend upon IL-4 as a differentiation stimulus (11–13). In quantitative terms, however, a more divergent picture is emerging. For example, in schistosomiasis IgG4 is found to peak early in the age profile of a human population, and to slowly decline relative to increasing IgE over time (14). The reversal of IgE:IgG4 ratios accompanies the expression of an age-related acquired immunity to invasive schistosomes (14). Less work has been carried out in filariasis, although recent data showed maximal IgG4 levels in childhood (15) whereas IgE levels were

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Table I
Summary of study population

Clinical Grouping	No.	Male	Female	Mean Age	Age Range
Asymptomatic, Mf-negative	47	29	18	33.7	15–65
Microfilaremic	45	41	4	39.0	15–64
Elephantiasis	55	46	9	46.5	15–75
Symptomatic, Mf-negative	17	11	6	42.1	19–56
Total	164	127	37	40.3	15–75

threefold higher in the over-15 age group than in the under-15 cohort (16).

The other Ig isotypes are of increasing interest in helminth diseases. Filariasis is characterized by edematous lesions that have been likened to type III hypersensitivity reactions, possibly mediated by C-fixing immune complexes (17, 18) for which the profile of isotype usage would exert a great influence. Conversely, the clearance of microfilarial parasites from the circulation has been strongly associated with IgM antibodies in animal models (19–21).

The majority of studies performed hitherto have involved relatively small numbers of subjects, or have concentrated at the qualitative level, searching for specificity correlates of the diverse states associated with filariasis. In this study, we set out to attain a comprehensive immunologic picture of a large sample in an endemic area, and we are currently determining cellular and humoral immune responses in a HLA-typed population from Indonesia. In this first report to emanate from the study, we describe the quantitative relationships between different isotypes of filarial-specific antibody, within the context of our current understanding of the basis for long term nematode infection and pathology.

Materials and Methods

Study population

The study population consisted of 164 residents of the Rengat district of Sumatra, Indonesia. This is an area endemic for zoonophilic (subperiodic) *Brugia malayi* (22), and the mf³ prevalence rate, assessed by Nuclepore filtration (Nuclepore Corporation, Pleasanton, CA) of 1-ml blood volumes, ranged in the nine villages surveyed from 10 to 15%. The purposes of the study and the procedures involved were explained to all residents, and only those granting informed consent were enrolled as study participants. Significantly fewer women volunteered to take part in the study (Table I). Individuals were categorized into four groups as follows: 1) asymptomatic and Mf-negative on Nuclepore filtration of venous blood, and with no history of filarial disease; 2) microfilaremic, on the basis of Nuclepore filtration; 3)

chronic obstructive disease, classified as elephantiasis; and 4) Mf-negative but with current or recent filarial symptoms (lymphadenopathy and/or pitting edema), and including individuals with microfilaremia in the previous year. A summary of the study group is given in Table I. Because the last grouping of symptomatic Mf-negative persons was quite disparate, and considerably smaller than the other categories, comparative analyses presented in this paper are restricted to the three main subsets of asymptomatic Mf-negatives, microfilareemics, and elephantiasis patients.

Between 20 and 40 ml of heparanized venous blood was taken from each patient and stored at ambient temperatures for up to 8 h during air transportation to Jakarta for separation of lymphocytes for cellular studies (23). An equal volume of RPMI 1640 medium was added and diluted blood separated over Ficoll into cellular and plasma fractions. Plasma samples were then recovered and stored at –70°C until required.

Parasite Ag

Somatic extract Ag was prepared as described elsewhere (24). Briefly, 100 adult *B. malayi* worms were crushed in 1.7 ml of ice-cold PBS-protease inhibitors (24) in a ground glass homogenizer. Then, 300 µl of 15% *n*-octylglucoside were added and the homogenate stood on ice for 60 min. After further brief homogenization, the suspension was centrifuged and the supernatant recovered, protein concentration determined, and aliquoted at –70°C.

ELISA for IgG isotypes

Adult *B. malayi* extract, diluted to 1 µg/ml in carbonate buffer, was coated onto Nunc microtiter plates at 200 µl/well. Plates were stored overnight at 4°C, and blocked before use in ELISA by a 30-min incubation in 5% FCS in Tris-buffered saline. Plasma samples were analyzed at a range of dilutions from 1/80. Patient plasma dilutions were incubated for 2 h at 37°C, and plates washed well before addition of isotype-specific mouse mAb (from Oxoid, Hampshire, UK) as follows: IgG1 (M15015, clone NL16, WHO/IUIS code HP6012) 1/4000; IgG2 (M10015, clone GOM1, WHO/IUIS code HP6008) 1/2000; IgG3 (M74011, clone and WHO/IUIS code HP6050) 1/1000; IgG4 (M11013, clone RJ4, WHO/IUIS code HP6011) 1/4000; IgM (M02012, clone AF6) 1/1000; and total Ig (M06014, clone 8a4, WHO/IUIS code HP6064), 1/4000. The isotype-specificities of the anti-IgG1, IgG2, IgG3, and IgG4 antibodies have been validated in independent studies (12, 25, 26).

After a further 2-h incubation, all wells were washed and received a 1/1000 dilution of peroxidase-conjugated rabbit-anti-mouse reagent that was devoid of reactivity to human

³ Abbreviations used in this paper: mf, microfilaria; SPRIA, solid phase RIA; BmA, *Brugia malayi* adult parasites.

Ig (Dako P260). Substrate (ABTS, 2,2'-azino-di[3-ethylbenzthiazoline sulfonate], Kirkegaard and Perry Laboratories, Gaithersburg, MD) was then added, and readings were taken at 20 min.

Each individual plasma was measured at a range of four or eight dilutions, and a titer calculated by interpolation or extrapolation on a regression line obtained with a standard high titer antiserum (E29); the minimum titer assigned was 1. Titers were converted to absolute measurements of IgG subclass concentrations by reference to a calibrated serum (82-17) made available by Dr. Eric Ottesen, National Institutes of Health, Bethesda, MD. The levels of specific anti-filarial antibody in E29 were found to be: IgG1, 48 µg/ml; IgG2, 182 µg/ml; IgG3, 48 µg/ml; IgG4, 1210 µg/ml. IgM levels were calculated as the titers that equaled a threshold value defined as a 1/3,200 dilution of the reference antiserum (E29).

SPRIA assay for IgE

B. malayi adult Ag was coupled on to cyanogen bromide-activated Sepharose CL-4B beads (Pharmacia, Uppsala, Sweden) at a ratio of 175 µg of Ag to 100 mg of Sepharose for a SPRIA. Coupled beads are referred to as BmA-Sepharose. Plasma samples, diluted 1/2 with RPMI 1640 medium, were depleted of IgG subclasses before the IgE assay by overnight incubation of 300 µl of diluted plasma with packed protein G-Sepharose 4 Fast Flow beads pelleted from 300 µl of suspension (Pharmacia 17-0618-01). Absorbed plasmas were recovered by centrifugation. The SPRIA assay detected from twice (elephantiasis) to four times (microfilariaemia) higher levels of IgE in samples depleted of IgG antibodies by protein G absorption.

The assay for specific IgE was conducted by incubating 0.5 mg of BmA-Sepharose with 50 µl of 1/10 diluted plasma, in a final volume of 300 µl of PBS-0.3% BSA-0.1% Tween (PBS-AT). After an overnight incubation shaking at room temperature, the samples were washed five times in PBS-0.1% Tween (PBS-T), the beads were mixed with 250 µl of PBS-AT containing 4.5% normal bovine serum and 0.5% normal sheep serum, and then received 20,000 cpm of a ¹²⁵I-labeled polyclonal sheep antiserum against IgE, which shows no binding to human IgG-Sepharose (CLB: 19-F1). The final volume was 550 µl. This was incubated overnight on an end-over-end rotator at room temperature. After four washes with PBS-T, the samples were counted in a gamma counter, expressed as % binding, and then plotted to a nonlinear regression curve of a reference serum. The reference curve was constructed by incubating Sepharose-coupled sheep anti-IgE (CB: 19-F2) with serial dilutions of a reference serum under identical conditions to those described above.

The determination of total IgE levels was conducted as follows. A reference serum containing 32,000 IU/ml of IgE

was used, from which 50 µl was coupled with 100 mg of CNBr-activated Sepharose CL-4B. These beads were used as the solid phase in a competitive RIA. For each point, 250 µl of Sepharose was incubated with 1 to 10 µl of plasma samples and 20,000 cpm of ¹²⁵I-sheep anti-IgE. After an overnight incubation, shaking at room temperature, samples were washed five times with PBS-T, counted in a gamma counter, and the % binding calculated. By means of a calibration curve with dilutions of a reference serum with known IgE content, the IgE levels of the study samples were calculated in IU/ml.

Statistical analysis

Geometric means and SD were calculated from logarithmically transformed data for all titers and antibody concentrations. For specific IgE antibody levels, in which 5/164 individuals were negative, these values were assigned threshold values (0.4 ng/ml) for the purpose of geometric mean calculation. Percentage and ratio data were calculated arithmetically for each individual. Arithmetic means are presented for the distribution of IgG subclasses, which add to 100%. Geometric means are presented for ratios of specific to total IgE, and of specific IgE to specific IgG4. Nonparametric statistical analysis was employed throughout with the Mann-Whitney U test.

Results

IgG subclass responses

The levels of each of the four subclasses of filarial-specific IgG antibodies were determined for all 164 patients in the study by ELISA on somatic extract of BmA. A range of dilutions was used for each serum, and threshold titers determined by reference to a standard antibody. These titers were then converted to absolute quantities (micrograms per milliliter) by normalization to a standard serum (provided by Dr. Eric Ottesen, National Institutes of Health) and the data obtained are presented in Table II.

The most striking feature of this subclass analysis is the predominance of IgG4 antibodies, normally only 2 to 5% of total IgG (25, 27). This finding in Brugian filariasis is very similar to that previously reported for Bancroftian filariasis (7). As can be seen from Table II, Mf carriers showed a mean filarial specific IgG4 level (762 µg/ml) at the higher end of the normal range for total serum IgG4 (27), whereas the mean level in elephantiasis patients was approximately threefold lower ($p < 0.001$). When the relative distributions of the four IgG subclasses were calculated, it was found that IgG4 constituted 57% of specific IgG antibody in elephantiasis patients, and in Mf-positive carriers the proportion averaged 88% (Fig. 1). It was noted that the asymptomatic Mf-negative ("endemic normal") group also showed elevated IgG4, making up 55% of the anti-filarial IgG response, but as will be described below

Table II
Levels of specific anti-filarial antibodies of each IgG isotype^a

	IgG1	IgG2	IgG3	IgG4
Asymptomatic, Mf negative (n=47)	12.7 (8.7)	1.7 (18.8)	3.7 (10.8)	25.3 (30.8)
Microfilaremia (n=45)	31.7 (2.5)	3.0 (13.1)	6.0 (9.8)	761.5 (2.8)
Elephantiasis (n=55)	75.4 (2.6)	39.7 (3.8)	36.7 (3.5)	221.8 (7.2)
Symptomatic, Mf negative (n=17)	34.1 (6.6)	3.2 (20.5)	11.8 (6.8)	81.0 (14.4)
Total study group (n=164)	32.9 (5.1)	6.1 (15.6)	10.3 (8.9)	150.4 (15.0)
Normal serum				
Mean	5,910	3,040	610	240
Range	3,190–10,200	1,230–6,630	160–1,940	30–1,330
Percentage	60.3	31.0	6.2	2.5

^a All values are in micrograms per milliliter. Data represent the geometric means (and geometric SD) from the indicated number of individuals. Values in brackets are the factor by which the mean is divided and multiplied to give the limits of 1 SD. Very high SD (e.g., for IgG4 in asymptomatic Mf-negatives) reflect a very heterogeneous distribution as presented in Figure 2. Values for normal serum IgG isotypes are taken from a study of 174 UK individuals [27]. Normal serum ranges represent the 95% confidence limits from the same study.

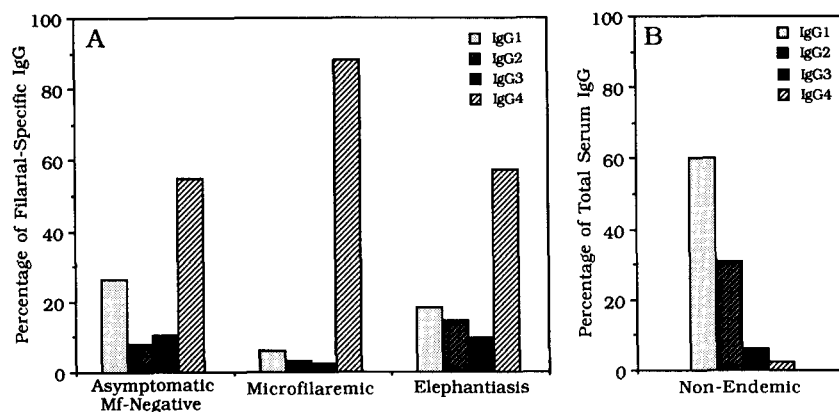


FIGURE 1. A, the distribution of IgG subclasses, expressed as percentage of filarial-specific IgG antibody measured against *B. malayi*, in three groups of patients. B, The average distribution of total serum IgG subclasses in normal individuals from filarial non-endemic areas (27).

there was wide variation between individuals in this group.

Compared with chronic disease patients, Mf⁺ carriers showed depressed levels of all other subclasses in both absolute (Table I) and relative (Fig. 1) terms. In contrast, IgG1, IgG2, and IgG3 antibodies were all high in elephantiasis, and in the study overall these isotypes showed positive correlations with each other ($r = 0.539$ to 0.670 , $p < 0.001$). IgM antibodies were also examined, and were found to be high in all cases. Indeed, significant IgM was found even in nonendemic control sera, presumably because of reactivity to phosphorylcholine and other nonprotein determinants (28, 29). IgM antibodies were not quantitated in absolute terms, but using an arbitrary threshold, chronic pathology patients had a mean titer of 5312, microfilaremics a titer of 4175, and endemic normals an intermediate value of 5060.

These average values conceal some interesting patterns of individual variation in isotype responses. Focusing on the distribution of IgG4 within each disease group, it was found that almost all of the Mf⁺ group had high levels (Fig. 2). Two measures fulfilled by 95% (43/45) of the Mf⁺ group were that they had greater than 100 $\mu\text{g/ml}$ of specific IgG4 antibody, and more than 70% of their specific IgG response was IgG4. This supports earlier claims that abnormally high specific IgG4 is diagnostic of an active filarial infection, based on work with *Wuchereria bancrofti* patients (8). By this criterion, the asymptomatic Mf-negative group (Fig. 2) may be considered as an amalgam of two types: a low ($<100 \mu\text{g/ml}$) IgG4 group who may not be currently infected, and a high ($>100 \mu\text{g/ml}$) IgG4 group who, we suggest, harbor a cryptic infection. Likewise, many but not all (41/55) of the patients with elephantiasis

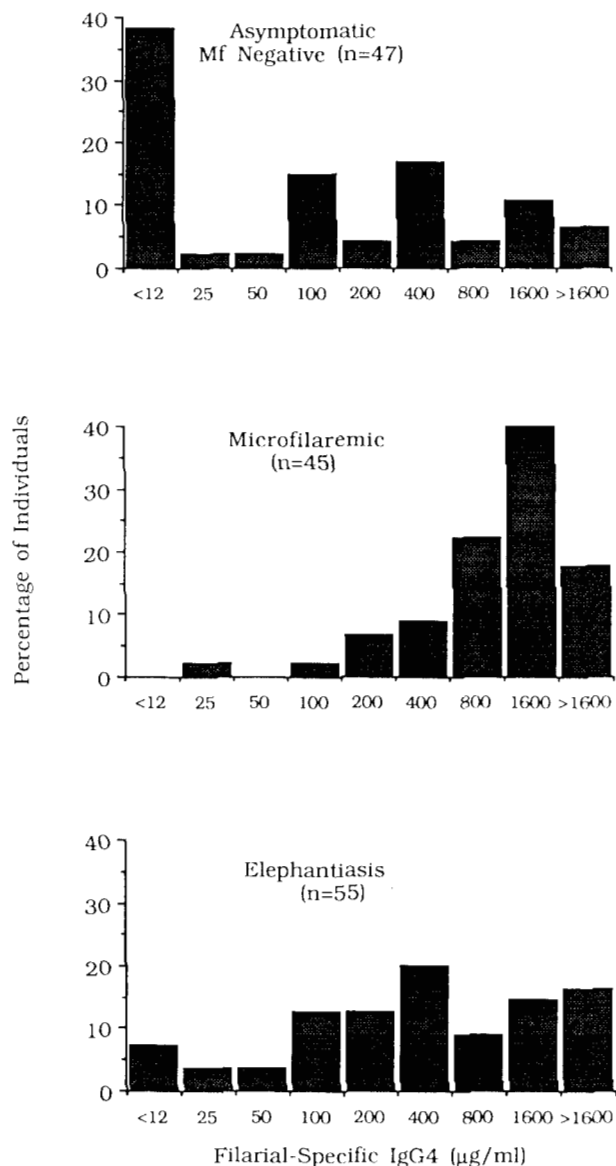


FIGURE 2. The distribution of filarial-specific IgG4 levels, in µg/ml, among individuals in the study population. Category numbers represent the upper limit of each range.

have high IgG4 and may be presumed to still be infected. The use of high IgG4 as a diagnostic indicator of infection with *B. malayi* is important as in this species assays for circulating filarial Ag have not been successful (30).

The levels of filarial-specific and total serum IgE were then measured. In contrast to the IgG4 pattern, IgE levels were higher in chronic pathology patients compared with the asymptomatic cases (Fig. 3 and Table III). Most notably, there was no significant correlation between IgE and IgG4 antibody levels ($r = 0.129$, $p = 0.1$) although specific IgE does correlate with all other IgG subclasses ($r = 0.373$ to 0.506 , $p < 0.001$). To illustrate the contrast observed, IgE values were plotted against IgG4 levels for each individual in the asymptomatic, microfilaremic, and elephantiasis groups (Fig. 4). Figure 4 shows that 60% (27/45) of Mf⁺

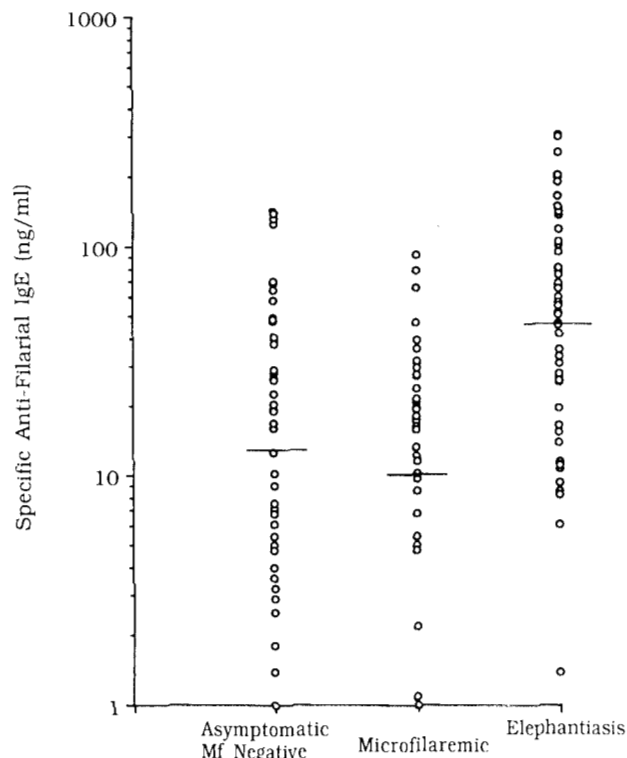


FIGURE 3. Levels of filarial-specific IgE in the study population, measured in nanograms per milliliter. Two asymptomatic Mf-negative and three microfilaremic individuals had zero IgE levels (threshold 0.4 ng/ml). Specific IgE in elephantiasis was significantly higher than either asymptomatic or microfilaremic groups (Mann-Whitney U test, $p < 0.0001$). There was no significant difference between the asymptomatic or microfilaremic subjects.

have above-median values of IgG4 and below-median values of IgE ($z = 9.46$, $p < 0.0001$). Only 5% of Mf⁺ but 40% of elephantiasis cases present the reverse relationship of high IgE and low IgG4.

Patients with chronic filarial pathology also bore a higher percentage of their total serum IgE with anti-filarial specificity (Table III), because although their specific IgE was four times higher than for microfilareemics, the total IgE was less than twofold higher. Thus, the density of anti-filarial antibody on mast cell and basophil membranes is likely to be significantly higher in elephantiasis.

IgG4 in particular is thought to act as a blocking antibody for IgE-mediated hypersensitivity reactions (31, 32). For this reason, the ratio of IgE to IgG4 was calculated for each individual, and the mean for chronic pathology patients (194 ng IgE/mg IgG4) was found to be some 10 times higher than that for asymptomatic microfilareemics (16.06 ng/mg), suggesting that IgE may mediate some pathogenic effects in disease, but that sufficient IgG4 levels may protect asymptomatic individuals. However, this ratio was even higher in asymptomatic Mf-negatives (349 ng/mg), demonstrating that high IgE with little IgG4 is not itself

Table III
Specific and total IgE levels^a

Patient Group	Specific IgE	Total IgE	% Specific
Asymptomatic Mf-negative (n=47)	11.84 (6.91)	917 (3.31)	0.63 (3.13)
Microfilaremic (n=45)	9.63 (7.75)	1155 (4.91)	0.39 (2.85)
Elephantiasis (n=55)	42.97 (3.08)	2010 (3.13)	0.89 (3.72)
Symptomatic Mf-negative (n=17)	28.50 (3.36)	1011 (4.53)	1.18 (4.61)

^a All values for IgE are in nanograms per milliliter, and represent the geometric means and SD of *n* individual patients. The values for percentages are the geometric means of individual percentages (specific IgE × 100/total IgE) from the same sets. For comparison, the values determined in a South Indian population infected with *W. bancrofti* by Hussain et al. [1] were 55 ng/ml in endemic normals (n=12); 30 ng/ml in microfilaremics (n=11); and 68 ng/ml in chronic pathology (n=52). These levels represented 3.2%, 0.86%, and 2.5% of total IgE, respectively.

sufficient to initiate pathology.

A major feature of filariasis, as with most other helminth infections, is a marked age-dependence in prevalence of infection and disease (33–35). Each antibody isotype was examined for changes with age in the study population, by classification into age groups. For all isotypes other than IgG4, a distinct U-shaped age profile was observed, with high levels in the youngest group (15 to 20 yr) being followed by a sharp decline in the 21- to 30-yr class before increasing again in successively older age groups (data not shown). For IgG4 a gradual decline with age was found. Although these differences did not attain statistical significance, they were in concordance with our other findings demonstrating that IgG4 is regulated separately from the other Ig isotypes.

Discussion

Filariasis captures many essential characteristics of helminth infections (36–38). The human immune response to filarial parasites shows a remarkable skewing of the humoral immune response toward IgE and IgG4 (1, 7, 8). Enhanced production of these antibody isotypes in humans is now known to be promoted by IL-4 (11–13), which in turn reflects selective activation of the Th2 subsets of Th lymphocytes (4, 39, 40). In this context, the study of isotype expression in filariasis can help explain immunologic events in this particular disease, and also indicate some new facets of the regulation of antibody synthesis that may prove worthy of investigation.

Individuals exposed to filarial transmission may be subdivided into asymptomatic microfilarial carriers with hyporesponsive or anergic immune status, symptomatic cases with strong immune responses and varying degrees of

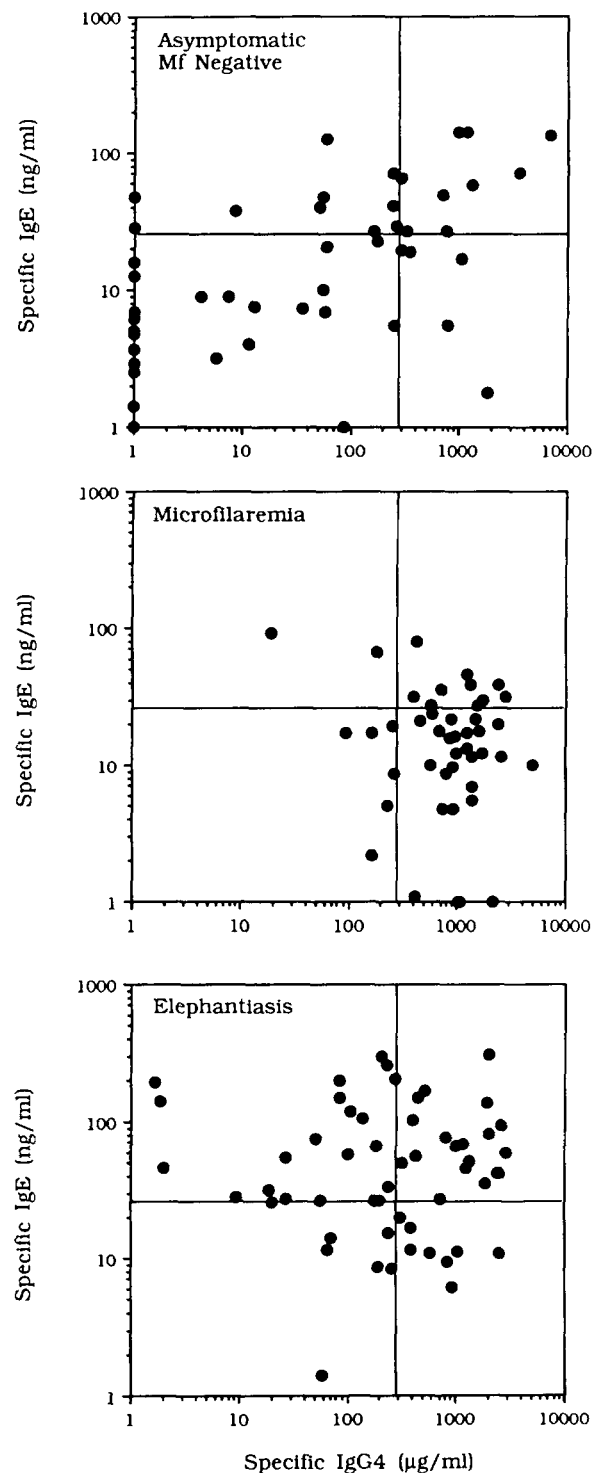


FIGURE 4. Relationship between serum levels of specific and IgG4 for the three main groups within the study. Each plot is divided by a horizontal line representing the median IgE value for the whole population (24.5 μg/ml) and a vertical line representing the median IgG4 value for the whole population (292 μg/ml).

pathology and few or zero circulating parasites, and subjects with no apparent sign of infection or disease: the so-called "endemic normal" that we term asymptomatic Mf-negative (41, 42). The concept of "endemic normal" is one of particular controversy in filariasis because many if not most of this group harbor cryptic infections (43). Indeed, in a recent study only a small proportion of endemic individuals could unequivocally be shown to be free of infection (44). The major aims of this study were therefore to examine differences between the responses of asymptomatic Mf-positive and elephantiasis patients, and to analyze the asymptomatic Mf-negative ("normal") group in an effort to improve our understanding of this spectral disease.

Our most immediate observation was that of extremely high IgG4 responses in all individuals in the patent microfilaremic group, corroborating previous reports that elevated IgG4 is a diagnostic marker for infection (8). Overall, *B. malayi* microfilareemics have a three times higher IgG4 level than cases of elephantiasis. Perhaps equally important is that in amicrofilaremic subjects (with or without filarial pathology), levels of IgG4 generally fit a bimodal pattern which may reflect the fact that a subset within these groups carries an active infection. Previous reports on Bancroftian filariasis have indicated a state of B cell anergy with total IgG levels in microfilareemics up to eightfold lower than in chronic filariasis (45). In this study, however, Mf⁺ patients show a higher total anti-filarial IgG titer as a result of a very substantial IgG4 response, although all other isotypes are depressed.

The IgG2 and IgG3 subclasses are considerably more prominent in individuals with elephantiasis than in asymptomatics. IgG3 has been implicated as a factor in onchocerciasis, in which individuals with Sowda (lichenified onchodermatitis characterized by low parasite loads and extensive tissue damage) are uniquely high in parasite-specific IgG3 (18), possibly implying a type III immune complex-mediated hypersensitivity in pathology. A similar link was suggested by Hussain et al. for lymphatic filariasis (17). IgG4 is functionally monovalent (9, 46), does not fix C (46), and may also block type III reactions as well as IgE-mediated type I. If so, the ratios of IgG4 to IgG3 may be a critical measure. An alternative protective role for IgG3 in onchocerciasis has been suggested, with a high IgG3:IgG1 ratio being most strongly associated with resistance (47). This finding is more difficult to compare with our study as we made no attempt to define "immune" individuals.

IgE expression within the study individuals also followed a distinctive pattern. The mean level in elephantiasis was four times higher than in asymptomatics, although exceptional asymptomatic individuals were found with high IgE as well as pathology cases with very low IgE scores. A survey of *W. bancrofti* filariasis also reported higher se-

rum IgE levels in clinical pathology cases than in asymptomatics (1). Moreover, in onchocerciasis, patients with the highest skin microfilarial densities tended to have the lowest specific IgE, and maximal IgE levels are seen in those with lowest Mf burden (48). These serologic assays have been more consistent than earlier results from skin testing filariasis patients for immediate type hypersensitivity reactions (49).

The results of this study raise some interesting possibilities concerning two closely juxtaposed issues: those of parasite killing and the generation of pathology. Active infection appears to promote IgG4, limit the level of IgE, and avoid stimulation of IgG2 and IgG3. The development of pathology, and the elimination of circulating parasites, is associated with higher IgE:IgG4 ratios and expansion of the other IgG subclasses. We cannot determine whether the change in isotype balance leads to killing and pathology or whether removal of parasites eliminates an immunomodulatory effect on class switching in antibody-producing cells. Studies on the change of isotype expression after chemotherapeutic removal of parasites would be instructive in this respect. IgE has often been considered as a protective isotype against helminths (50), and this is supported by data from a rodent filariasis model (51). Moreover, in human schistosomiasis high IgE responders and low IgG4 responders are 10 times less likely to be infected than those with reciprocal responses (14).

Maximal IgG4 responses in humans have generally been associated with repeated stimulation, both by pathogens such as schistosomes (52–55) as well as by certain non-infectious agents (9). However, the gradual decline in IgG4 levels with age, with no associated reduction in exposure, implies that mature rather than infective stages of the parasite are responsible for promoting IgG4. Consistent with this hypothesis, we have previously reported in a study on Bancroftian filariasis that IgG4 levels correlate significantly with microfilarial density (8).

What underlies this differential regulation of IgE and IgG4? Similar distinctions between these isotypes have been reported in diverse conditions, including *Trichinella spiralis* nematodiasis (56), schistosomiasis (14, 57), and in allergic patients in whom a switch from IgE to IgG4 was observed after desensitization therapy (58). IL-4 is a central player in this system, emanating from Th0- and Th2-like T cells, from mast cells, and from some CD8 cells. The absence of a Th1 cell is implied by the relatively low T cell proliferative responses engendered in asymptomatic microfilareemics (23). Although IL-4 is a prerequisite for both IgG4 and IgE class switching (11, 13), costimulation of the B cell surface receptor CD40, normally delivered by activated T cells, is required in both cases (59, 60). The circumstances in which IgE secretion may be blocked without inhibition of IgG4 have not been defined, although such an

effect can be achieved by the pharmacologic mediator disodium cromoglycate (61). It is possible therefore that the selective production of IgG4 in filariasis reflects the ability of microfilarial parasites to produce a mediator of similar nature. One such product known to be secreted by microfilariae is PGE₂ (62) which has also been reported to inhibit IgE but not IgG4 synthesis by human B cells (61).

This study has revealed a number of important facets of the immune response and its relationship with filarial infection and disease. Our data raise the important question of whether IgE-mediated reactions contribute to the pathology of filariasis and whether IgG4 acts to block pathogenetic processes. We are now extending analyses of specific Ag recognition, with complementary cellular and genetic investigations on the same individuals, to determine specific T cell responsiveness to total and individual filarial Ag, and the relationship between MHC class I and II alleles and the course of infection in different subjects. These studies will be reported in a subsequent paper (23).

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