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Helminth infections, allergic disorders and immune responses: studies in Indonesia

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Citation

Wahyuni, S. (2006, November 22). *Helminth infections, allergic disorders and immune responses: studies in Indonesia*. Retrieved from <https://hdl.handle.net/1887/4986>

Version: Corrected Publisher's Version

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Note: To cite this publication please use the final published version (if applicable).

Chapter 3

Anti-filarial and total IgG4 and IgE
antibody levels are correlated in mothers
and their offspring

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Transactions of the Royal Society of Tropical Medicine and Hygiene
2002; 96(3): 231-238

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Abstract

In mothers who suffer from helminth infections or allergic diseases, prenatal sensitization with antigens/allergens is suspected to bias the immune system of the offspring towards a T helper (Th)2 type response. To investigate this at the antibody level, we collected 113 blood samples on filter paper from a paediatric population aged 3 months to 10 years and their mothers, who resided in an area endemic for brugian filariasis in Indonesia. The results showed that antibody levels in children were strongly correlated with maternal antibody levels. However, for anti-filarial immunoglobulin (Ig)G4 and IgE this relationship was manifested directly after birth, whereas for total antibody levels a positive correlation could be detected with children aged two years and older only. To investigate the influence of paternal antibody on progeny, specific IgG4 was determined in a different set of samples from 229 children and both of their parents. Interestingly, the influence of paternal IgG4 became apparent after the age of four years only. In contrast, maternal antibody levels were correlated to levels produced by their offspring at young age already (3 months onwards). Taken together, it appears that children can become sensitised to parasite antigens intrauterine, allowing them to produce Th2-dependent specific IgG4 and IgE antibodies at young age, whereas with increasing age, the influence of environmental factors, shared in households, such as filarial transmission and other helminth infections, becomes dominant.

Introduction

In recent years, prenatal sensitisation has received much attention as it is suspected to predispose the neonate to infectious diseases, such as helminths, or to immunological disorders, such as atopy. Atopic disorders and helminth infections have in common a strong skewing of the immune response towards Th2 with the overproduction of Th2-cytokines (interleukine (IL)-4, IL-5 and IL-13) and IgG4 and IgE antibodies, subclasses that are otherwise tightly regulated [297;306;307].

Evidence for a role of prenatal sensitisation in development of disease and infection has come from several studies. In the field of allergy, it was shown that although low Th2 responses were present in both atopic and non atopic children at birth, there was a rapid suppression of Th2 responses during the first year of life in non-atopic children, in contrast to consolidation of responses (associated with defective interferon- γ production) in atopics [308]. These responses in atopic children may be enhanced by maternal exposure to relevant allergens during pregnancy, indicating that antenatal immune modulation is crucial for the development of atopy [309]. In areas endemic for schistosomiasis and filariasis, it was demonstrated that the human foetus develops similar patterns of cytokine production as observed in mothers [310]. In addition, cord blood (CB) lymphocytes of new-borns producing helminth-antigen driven B-cell responses were more likely to be from schistosome-

or filaria-infected mothers than from uninfected mothers [311]. Furthermore, several studies have reported the synthesis of IgE in the unborn foetus by detection of allergen- or filaria-specific as well as total IgE antibody in CB [195;201;311-313].

Despite the abundant information on the influence of specific antigens or allergens on sensitisation of neonates, little is known about the relationship between specific and total antibody levels of the mother and humoral immunity in offspring. The present cross-sectional study was designed to investigate whether maternal antibody levels are related to specific and total IgG4 and IgE antibody responses of their children. In lymphatic filariasis, specific IgG4 antibodies are dependent upon chronic exposure to parasites and can be used as an immunological marker for indicating active filarial infection [73;305;306]. We have collected 113 blood samples on filter paper from a paediatric population aged 3 months to 10 years and their mothers, who resided in an area endemic for *Brugia malayi* in Indonesia. To establish the influence of paternal antibodies as well, a different set of samples was collected from 229 children and both parents residing in a closely situated area to investigate the association between IgG4 antibody levels in parents and offspring.

Materials and Methods

Description of the study sites

The study was conducted in Mamuju Regency in South-Sulawesi, Indonesia, which is endemic for periodic nocturnal *B. malayi* [40;314]. Study A took place in Budong-budong district; villages Kire, Tumbuh and Karondang were situated at distances of approximately 10-15 kilometres from each other, but showed variable *B. malayi*-microfilaria (mf) prevalences in adults (20 years and older) of 5.6% (7/125), 23.1% (9/39) and 41.5% (44/106) respectively. Study B was performed in district Karossa, in villages Salubarana and Kalia, which were situated at a distance of 8 kilometres, with mf prevalences of 9.4% (29/309) and 9.6% (19/198) respectively.

In cooperation with the head of the village, local medical doctor and schoolteachers, parents and children were invited to participate in the study. Door-to-door surveys were performed to collect blood from parents and young children. Filter paper blood from children visiting primary school was collected at school. Informed consent was obtained from parents in accordance with the guidelines of Indonesian Department of Health and Human Services.

In study A, 54 mothers and 113 of their children (aged 3 months-10 years) took part; mean age of mothers was 28.9 years (range 18-45) and average number of children per mother was 2.1 (range 1-5). Table 1, shows the age and sex distribution of the children.

Age	Number of subject	M/F	Specific IgG4		Specific IgE		Total	
			mean	prevalence*	mean	prevalence*		
0-2	28	16/12	3.8	7%	3.7	14%	5.3	2.8
3-4	31	15/16	4.1	23%	4.0	39%	5.9	3.4
5-6	17	6/11	4.2	29%	4.2	59%	6.1	3.8
7-8	25	11/14	4.5	64%	4.2	68%	6.1	3.8
9-10	12	6/6	4.5	50%	4.3	92%	6.2	3.8
Total	113	54/59	4.1	32%	4.0	48%	5.9	3.5
Mothers	54		4.2	44%	4.1	57%	6.1	3.5

Table 1. Description of the study population A. Number of study participants, male/female distribution, mean levels and prevalence of specific IgG4/IgE, and means levels of total IgG4 are given for different age groups of children and their mothers. (Note that mean antibody levels are expressed as log₁₀ values.)

*Prevalence is determined by percentage of individuals showing reactivity above mean + 3SD of 20 European donors

As we had no information about their fathers and chemotherapy was already administered, we started a new study to obtain data of both parents. In study B, 229 children participated (aged 2 months-10 years). The mean age of mothers was 30.7 years (range 19-55), the mean age of fathers was 36.7 (range 20-76) and the average number of children per parent 2.1 (range 1-5). A description of the study population of study B is given in table 2.

Age	Number of subjects	M/F	Specific IgG4	
			mean level	prevalence*
0-2	47	24/23	3.6	13%
3-4	51	29/22	3.7	24%
5-6	53	36/17	3.9	23%
7-8	45	24/21	4.0	27%
9-10	33	20/13	3.8	18%
Total	229	133/96	3.8	21%
Mothers	110		4.0	37%
Fathers	112		4.7**	67%**

Table 2: Description of the study population of study B. Number of participants included in the study, male/female distribution, mean levels and prevalences of specific IgG4 are given for different age groups of children and for mothers and fathers.

* Prevalence is determined by percentage of individuals showing reactivity above the mean + 3SD of 20 European donors

** Significantly higher than mothers, $p < 0.001$

Blood collection and storage

Blood samples were collected during daytime on Whatman No. 3 paper strips of 1 by 2 cm, by pricking the finger with a sterile lancet. Care was taken to ensure that the blood penetrated to the reverse side of the paper. The papers were air-dried and stored in self-sealing plastic bags with silica gel at 4°C for a period of 10 months.

Elution of dried blood spots

Elution of filter paper blood samples was performed as described before [315]. For detection of specific and total antibody, filter paper samples were eluted overnight (ON) in assay buffer (0.1 M Tris-HCl (Ph 7.5) 0.05% Tween-20). For measurement of anti-filarial IgG4, a different assay buffer was used (phosphate buffered saline (PBS), containing 5% foetal calf serum (FCS) and 0.05% Tween-20).

Parasite antigen

Adult *B. malayi* worms were purchased from TRS labs, Athens, Georgia, USA. Female worms were freeze dried, ground to powder, dissolved in PBS, homogenised and slowly stirred ON at 4°C. The protein concentration was determined by 2,2'-biquinoline -4,4'-dicarboxylic acid disodium salt hydrate (BCA) method before storage at -20°C.

Enzyme-linked immunosorbent assay (ELISA) for detection of IgG4 and IgE

Detection of anti-filarial IgG4 and IgE was performed in ELISA technique, which has been described in detail before [316]. Optical density (OD) values of patient plasma were converted into arbitrary units (A.U.) by drawing a standard curve of plasma from a *B. malayi* positive donor in Central Sulawesi, Indonesia. A cut-off value for *B. malayi* antigen (BmA)-specific IgG4 and IgE antibodies was determined by taking the mean IgG4 reactivity (A.U.) plus three times standard deviations of 20 healthy Dutch donors at the Blood Bank in Leiden.

For detection of total IgE and IgG4, microtitre plates were coated ON at 4°C with 100 µl/well of either rabbit anti-human IgE (ε-chains, DAKO, Denmark) or mouse anti-human IgG4 (clone HP 6023, Sigma, St. Louis, USA) at 1/1000 dilution in 0.1M carbonate buffer (pH 9.6). Plates were blocked for 1 hour at 37°C with 100 µl/well PBS containing 2% bovine serum albumin (BSA, Albumin Fraction V, Boehringer, Mannheim, Germany). For measurement of IgE, filter paper eluates were tested at dilution 1/2 and 1/4 in assay buffer at 100 µl/well (corresponding to serum dilutions 1/16 and 1/320). A positive standard serum containing human IgE (NIBSC, Potters Bar, UK) was diluted down 1/2 in a series from 200 ng/ml until a final concentration of 0.195 ng/ml on each plate. After washing, IgE biotinylated goat anti-human IgE antibodies (ε-chains, 0.5 mg/ml, 1/1000 (Vector Laboratories, CA, USA)) were added followed by AP-conjugated streptavidin (diluted 1/3000 (Boehringer Mannheim, Germany)). For detection of total IgG4, filter paper eluates were diluted 1/1500 and 1/4500 in assay buffer (equal to serum dilutions 1/1.2*10⁵ and 1/3.6*10⁵). A positive standard serum containing human IgG4 (CLB, Amsterdam, the Netherlands) was incubated in a dilution series as described for IgE. Subsequently, 100 µl of AP-conjugated goat anti-human IgG (1/9000 (Jackson, Pennsylvania, USA)) was added. Conjugates were all diluted in assay buffer and incubation steps were performed for 1h at 37°C while shaking. The colour was developed by addition of para-nitrophenylphosphate substrate (*p*-NPP (Boehringer Mannheim)) diluted in diethanolamine buffer (DEA, 0.5 mM MgCl₂, 0.1 M DEA, pH 9.6 (Merck, Germany)) and OD was measured at 405 nm after 30 minutes on a Microplate Autoreader (Bio-Tek Instruments).

Statistical analysis

Statistical analysis was performed in SPSS for Windows version 8.0. For analysis of specific IgG4 and IgE levels a log 10 transformation was used to obtain normally distributed data; in this paper specific or total IgE/IgG4 are always presented as transformed log 10 antibody level. The relationship between antibody levels in parents and their offspring was investigated by calculating Spearman's rank correlation. The t-test was performed to compare IgG4 or IgE antibody levels between two groups. *Chi-square* test was used for comparison of proportions. Multiple regression analysis was carried out on specific and total anti-filarial IgG4 and IgE levels to assess the impact of age, gender and filarial endemicity (estimated by mf prevalence of the village) and antibody levels of the mother on the specific and total IgG4 and IgE levels (in A.U.) in children. Various transformations of age and mf prevalence of the village were modelled (e.g. linear, natural logarithm, quadratic, square root) before selection of the natural logarithm (ln) of age and mf prevalence of the village (ln(mf prevalence)) in the final models, which provided the best fit.

Results

Study A: Specific and total IgG4 and IgE in children and their mothers

To investigate the relationship between maternal antibodies and IgG4 and IgE in their offspring, a set of 171 filter paper samples was collected. Both anti-filarial and total IgG4 and IgE antibodies of mothers (n=54) and their offspring (aged 3 months-10 years, n=113) were measured. As shown in table 1, specific and total antibody levels were lowest in infants up to two years and gradually increased with age. Figure 1 shows a positive association between maternal and child antibody levels for specific IgG4 (a) and IgE (b). This correlation was statistically significant both for young children up to two years of age ($\rho=0.54$ for IgG4, $p<0.01$ and $\rho=0.53$ for IgE, $p<0.01$) and children aged three years and older ($\rho=0.33$ for IgG4, $p<0.01$ and $\rho=0.45$ for IgE, $p<0.001$). When considering total IgG4 and IgE, there was a positive correlation between levels detected in children aged 3-10 years and their mothers ($\rho=0.30$ for IgG4, $p=0.01$ and $\rho=0.46$ for IgE, $p<0.001$). However, when analysing younger children (0-2 years), no correlation with maternal antibodies could be found ($\rho=-0.05$ for IgG4, $p=0.81$ and $\rho=0.13$ for IgE, $p=0.50$).

To control for age and other factors that might confound comparison, such as gender and filarial transmission intensity of the village, multiple linear regression models were constructed to evaluate the influence of maternal antibody levels on antibodies produced by children. Table 3 shows the goodness-of-fit of the four models and the regression coefficients with corresponding p-values of independent determinants, taken all children together. The association between maternal antibodies and levels of specific and total

IgG4 and IgE detected in children was confirmed by the analysis. Note that specific IgE and both total IgG4 and IgE levels were significantly higher in male than in female offspring. Similar to previous reports, there was a positive association between filarial transmission intensity of the village and specific IgG4 levels [305;307;317-319], whereas total IgE levels were down regulated in villages with higher filarial transmission.

Antibody level of child	Age	Sex	MF prevalence	Maternal antibody	R ² _{adjusted}	F [*]
Specific IgG4	0.32 (<0.001)	NS	0.21 (0.002)	0.25 (0.001)	0.33	14.8 (<0.001)
Specific IgE	0.30 (<0.001)	0.14 (0.06)	NS	0.59 (<0.001)	0.46	24.4 (<0.001)
Total IgG4	0.43 (<0.001)	0.14 (0.05)	NS	0.46 (<0.01)	0.48	26.9 (<0.001)
Total IgE	0.48 (<0.001)	0.28 (<0.001)	-0.12 (0.02)	0.36 (<0.001)	0.56	36.6 (<0.001)

Table 3: Multiple linear regression models for specific and total IgG4/IgE antibody levels of children (study A). Regression coefficients and p-values (in brackets) of dependent determinants Inage, sex, ln(microfilaria prevalence of the village) and corresponding maternal antibody levels are given. Sex was defined as follows: males=1, females=0. NS= not significant at the 10% level. (* For all models: n=113, df= 4,108).

Study B: Influence of parental antibody levels on offspring

Since fathers were not included in study A, we determined anti-filarial IgG4 levels in a different set of filter paper samples from 229 children residing in two *B. malayi* endemic villages, where both parents were included (n=222). As shown in table 2, specific IgG4 increased with age and reached a plateau in children older than 5 years. mf prevalence and antibody levels were significantly lower in mothers (mf prevalence=4%, mean specific IgG4=4.0) than in fathers (mf prevalence=14%, mean specific IgG4 =4.7; p =0.01 and p<0.001, respectively), which may reflect a down-regulation of filarial infection in women of reproductive age as reported in earlier studies [320]. All but five children (98%) shared the same residence with both parents.

Figure 2 shows the specific IgG4 levels of children in different age groups and their mothers (a) or fathers (b), which were either IgG4-positive or IgG4-negative. In all age groups, except 5-6 years, anti-filarial IgG4 levels in offspring were clearly influenced by

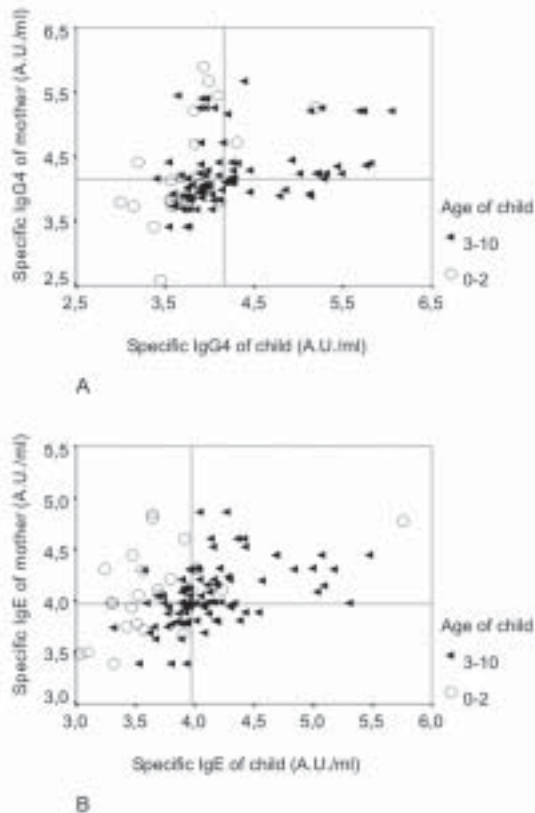


Figure 1: Anti-filarial IgG4 (a) and IgE (b) in mothers and their young (0-2 years) and older children (3-10 years) (study A). The straight lines represent the cut-off values for specific IgG4 (A) and IgE (B) reactivity. (Note that antibody levels are expressed as log₁₀ values).

the absence or presence of filarial infection in the mother, as reflected by specific IgG4. No association between IgG4 levels of fathers and offspring was observed in young children up to four years (mean IgG4=3.66 in children with IgG4-positive fathers, mean IgG4=3.67 in children with IgG4-negative fathers, $p=0.97$), in contrast to the influence of maternal IgG4 (mean IgG4=3.86 in children with IgG4-positive mothers, mean IgG4=3.53 in children with IgG4-negative mothers, $p=0.02$). However, in older children, aged five years and up, the specific IgG4 level of children with IgG4-positive fathers (mean IgG4= 3.96) was significantly higher than in children with IgG4-negative fathers (mean IgG4=3.70, $p =0.04$).

The correlation coefficients between specific IgG4 antibody levels of parents and their offspring showed that both maternal and paternal IgG4 levels were correlated with levels of their offspring when considering all children ($\rho=0.20$, $p<0.01$ for mothers and children, $\rho=0.16$, $p=0.01$ for fathers and children). In children up to four years of

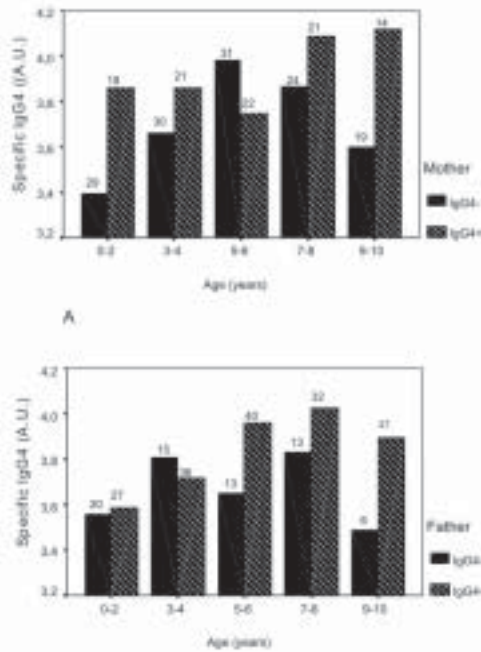


Figure 2: Anti-filarial IgG4 in children of different age groups born to IgG4-negative (dark bars) and IgG4-positive (hatched bars) mothers (A) or fathers (B) (study B). The number of subjects in age groups is given above each bar. (Note that antibody levels are expressed as log 10 values.)

age there was a significant correlation between antibody levels of children and their mothers ($\rho=0.25$, $p=0.01$), whereas no correlation between children and paternal IgG4 levels was observed ($\rho=0.06$, $p=0.59$). In children aged five years and older antibody levels were significantly correlated with antibody profiles produced by both parents ($\rho=0.18$, $p=0.04$ for mothers and children, $\rho=0.23$, $p=0.01$ for fathers and children). These data were confirmed by multiple linear regression analysis, controlling for age and gender of the child (data not shown).

Finally, the relationship of IgG4 antibody levels between both parents was investigated, and it was shown that, despite higher infection levels in fathers, specific IgG4 levels of mothers and fathers were positively correlated ($\rho=0.26$, $p<0.001$, $n=112$), suggesting a role for environmental factors on outcome of filarial infection.

Discussion

We set out to study how parental total and specific antibodies are related to immunoglobulins in their children during early and later childhood, and investigated filarial specific IgG4 and IgE as well as total IgG4 and IgE. Despite the lack of information on the immune status of parents at the time of birth, the stability of infection patterns over time in adults residing in endemic communities [321] would suggest a relative

stability of antibody patterns as well, as these are important in determining outcome of filarial infection [73;297;322].

The data showed that specific IgG4 and IgE antibody levels in mothers and their children up to 10 years of age were correlated. In contrast, when considering total IgG4 and IgE, maternal antibodies were associated with those of their offspring, if children were aged 3 years and older only. Although specific IgG4 levels produced by the father were correlated to IgG4 produced by their offspring, this relationship was only present in children aged five years and older, whereas the positive correlation between maternal and child's specific IgG4 antibody levels was present directly after birth.

Given the lack of an association between specific IgG4 antibody levels in fathers and young children, in the presence of a strong association between mothers and children, it seems likely that specific IgG4 and IgE production in subjects aged four years or less is determined by prenatal sensitisation. It is known that parasite antigens, maternal anti-idiotypic antibodies and even mf might cross the placental barrier intrauterine [323-325]. Several studies in CB have indicated that the unborn foetus is able to produce specific and total IgE antibodies after sensitisation [195;198;311-313]. In lymphatic filariasis evidence for prenatal sensitisation has come from studies in which CB lymphocytes from offspring of infected mothers residing in an area endemic for schistosomiasis and filariasis were able to produce helminth-antigen driven cytokines and IgE antibodies at birth [310;311].

Although we cannot exclude the possibility that specific IgG4 or IgE antibodies detected in infants were derived from their mothers, we consider this option unlikely. The fact that in the present study both specific IgG4 and IgE in young children were correlated with maternal antibody levels, whereas IgG4 and IgE of other specificities were unequally distributed in mothers and their progeny, makes it unlikely that only parasite specific antibodies were selectively transferred from mothers' milk during lactation. Moreover, several other arguments can be proposed against maternal origin. IgE antibodies are not able to cross the placental barrier [326] and maternal IgG antibodies transferred intrauterine will disappear from the neonatal circulation between 3 and 6 months after birth [327]. Furthermore, IgG subclass antibodies are abundantly present in colostrums and will be presented in the neonates circulation after absorption by the gut, but it remains less clear whether maternal IgE is present in breast milk and whether these antibodies can be absorbed in immunologically intact form [328-332].

Despite the lack of relationship between IgG4 antibody levels of fathers and young children, the data showed that specific antibody levels produced by children aged five years and older were equally correlated to paternal and maternal IgG4 levels. Furthermore, there was a significant correlation between specific IgG4 levels of mothers and fathers,

suggesting that environmental factors shared by a household play a role in the development of filarial infection and immune reactivity to these parasites. These data shed an interesting light on the factors that might regulate antibody production in children. The differential results obtained by children of various ages can be reconciled by proposing that in young children antibody production is initially determined by prenatal factors, whereas after the age of four years environmental or hereditary determinants overrule regulation of antibody production.

The influence of environmental and genetic factors on aggregation of parasitic infections has been demonstrated in a variety of helminth infections such as schistosomiasis, filariasis and strongyloidiasis [333;334]. Recently, genome wide scanning has led to the identification of a major locus involved in controlling parasite load in *Schistosoma* infections [145]. In lymphatic filariasis evidence for clustering of microfilaraemia within families and households has come from epidemiological studies [151;158;159], and recently we have demonstrated that specific IgG4 antibody levels are influenced by genetic, household and/or environmental factors [160].

The present data are in agreement with a recent study from a large data set in India, which rejected the hypothesis that prenatal sensitisation plays a role in determining outcome of filarial infection during childhood [151]. It was concluded that parental (and not only maternal) microfilaraemia increases the risk of infection in children aged five years and older, leaving the possibility that prenatal sensitisation could play a role in infection outcome at an early age only.

Corroborating our previous findings in a larger population of children residing in the same villages, the present data showed that specific IgE as well as total IgG4 and IgE levels were significantly higher in boys than in girls [316]. Although the mechanism and relevance behind this gender-related difference remains unclear, the findings are in agreement with a higher concentration of allergen-specific and total IgE in boys, which have been reported by a number of studies in the field of allergy [198;200;335].

In conclusion, we have demonstrated a strong association between filaria-specific and total IgG4 and IgE antibody levels in mothers and their offspring in children up to 10 years of age. The presence of a correlation between specific IgG4 antibody produced by young children with maternal antibody levels and simultaneous absence of an association with paternal antibody levels, are suggestive of a role of prenatally determined factors in antibody regulation during early childhood. However, since in older children the association of IgG4 antibody with levels produced by both parents is more or less equal, the influence of intrauterine sensitization seems to be dominated by environmental and/or genetically determined factors in later childhood.