Adults acquire filarial infection more rapidly than children: a study in Indonesian transmigrants

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SUMMARY

To dissociate the influence of host age from length of exposure on acquisition of filarial infection, we examined the development of microfilaraemia and anti-filarial IgG4 in all ages of a naive population that became suddenly exposed to *Brugia malayi* as a result of transmigration. Responses in 247 transmigrants, who had settled for periods of several months up to 6 years in their new homesteads, were compared with those of 133 life-long residents. As shown in earlier studies, anti-filarial IgG4 increased with age in the indigenous population, whose age is equivalent to length of exposure. However, by examining transmigrants, it became clear that development of specific IgG4 was influenced by age, since levels of this antibody were consistently higher in transmigrant adults than in transmigrant children, despite an equal length of exposure to filarial infection. Examining microfilaraemia, it was confirmed that infection establishes more rapidly in adults than in children.

Key words: lymphatic filariasis, IgG4, age, exposure, transmigrants.

INTRODUCTION

In areas endemic for lymph dwelling nematodes of *Wuchereria* or *Brugia* species, filarial infections are persistent and result in a spectrum of clinical manifestations that depend on several factors. *In utero* exposure to filarial parasites (Weil *et al*. 1983; Lammie *et al*. 1991; Steel *et al*. 1994; King *et al*. 1998), genetic make-up of the host (Ottesen *et al*. 1981; Chan *et al*. 1984; Yazdanbakhsh *et al*. 1995) and, in addition to these pre-determined factors, age or increasing exposure can influence acquisition of filarial infection and development of pathology (Day, Gregory & Maizels, 1991*a*; Day *et al*. 1991*b*). In a recent study we have demonstrated that reactivity to filarial antigens begins early in life (18 months) as measured by anti-filarial IgG4 and IgE and is differentially influenced by gender and transmission intensity (Terhell *et al*. 2000). Other studies have shown that the intensity of infection levels off with age or even in some cases declines in older age groups, which may indicate acquired immunity (Maizels *et al*. 1993). Longitudinal measurement of circulating antigens in children versus adults has shown that whereas children acquire new infections, adults with low or high worm burden resist new incoming larvae (Day *et al*. 1991*b*). One way to interpret these data is that with increasing exposure to filarial infection, immunity develops to larval stages. However, as the immune system matures and evolves with increasing age (Wade, Green-Johnson & Szewczuk, 1988; Horan & Ashcroft, 1997), the observed changes in filarial infection over years of exposure (from childhood to adulthood) may be caused by an age-dependent mechanism that is not necessarily exposure driven. With the increasing interest in the role that innate immune mechanisms play to control infection (Fearon & Locksley, 1996), understanding of how host age *per se* may influence acquisition of infection becomes relevant to defining components which contribute to immunity.

Most studies on the dynamics of filarial infection have been carried out in populations that are life-long residents of endemic areas. In such situations, history of exposure to parasites cannot be dissociated from age-related variation in immunity to filarial infection. This is only possible when a naive population of all ages becomes exposed to filarial infection abruptly. In Indonesia, the governmental transmigration programme relocates groups of people from overcrowded islands such as Java and Bali to under-populated and less-developed areas on more remote islands, which are often endemic for a

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multiplicity of infections. This transmigration policy provides a unique situation that allows the study of development of infection and immunity in individuals with varying age but with an equal length of exposure to infections.

The objective of the present study was to investigate the acquisition of infection with increasing length of exposure in new settlers, by measuring not only microfilariae in night blood but also by examining anti-filarial IgG4, which is an immunological marker of active filarial infection and has increased sensitivity compared to classical parasitological assays (Ottesen *et al*. 1985; Kurniawan *et al*. 1993; Haarbrink *et al*. 1995). We have studied 6 transmigration units in South-Sulawesi, Indonesia, which were established between 1990 and 1996 and 2 neighbouring villages of native Sulawesians in a region endemic for *B. malayi*. Both children and adults were included in the study in an attempt to dissociate the influence of age from exposure in development of filarial infection.

MATERIALS AND METHODS

Description of the study area

The survey was conducted in Budong-budong, a district of Mamuju Regency in South-Sulawesi, Indonesia, which is endemic for periodic nocturnal *B. malayi* (Slee, 1930; Partono *et al*. 1977). The transmigrants had travelled to their new homesteads in groups coming from the same village or region in Bali or Lesser Sunda islands as part of the government-sponsored relocation programme. Each year a new settlement was founded close to the former one (between 10 and 20 kilometres) which accommodated groups of transmigrants from 2–3 different regions together with migrants from Polmas, an over-populated area in South Sulawesi, to promote integration of different tribes. A total of 6 transmigrant units, settled between several months and 6 years prior to the survey, were included in the study together with 2 villages of indigenous Sulawesians, which were situated closely to the transmigration areas. Transmigrants from areas where filariasis is endemic (South Sulawesi and Lesser Sunda islands (Joesoef & Cross, 1978) were excluded from the analysis.

In co-operation with the medical doctors and health workers of the local District Health Centre and the head of each transmigration unit or village, all residents were informed about the study and invited to participate. Informed consent was obtained from all study participants or parents of underage children before parasitological studies and blood withdrawal in accordance with the guidelines of the Indonesian Department of Health and Human Services.

A total number of 247 transmigrants and 133 lifelong residents (LLR) were enrolled in the study. Transmigrants were grouped together according to year of arrival in the new area. Table 1 shows the number of cases, number of children (aged $\leqslant 15$ years) to adults (aged 16 years and older), sex distribution and microfilaria prevalence of transmigrants (according to length of residence) and indigenous Sulawesians. The number of children that participated in the survey varied per village and more males than females registered for the study. However, the average age of transmigrant children (range 6–15, mean = 10·2) was comparable to the indigenous children (range 4–15, mean = 10·4), and the same was true for adults in the transmigrant (range 16–70, mean = 32·6) and indigenous study population (range 16–60, mean = 31·2). For logistic reasons blood was collected during daytime in 2 villages, therefore microfilaria counts are lacking in a subset of study participants. Due to constraint of material microfilaria prevalence in the 3 year residents could be determined in 20 out of 67 study participants only. After parasitological examination and blood withdrawal all individuals with circulating microfilariae were treated with a 12-day course of diethylcarbamazine (DEC).

Blood collection

Venous blood samples of 10 ml were collected between 20.00 h and 24.00 h. Ethylenediaminetetraacetic acid (EDTA) was added at a final concentration of 0·05 M. The tubes were centrifuged and plasma was stored at −20 °C for several months until shipment to the Netherlands, where it was stored at −70 °C until use.

Parasitological examination

After centrifugation and removal of plasma, 10 ml of distilled water was added to the remaining blood pellet and was shaken firmly. The next day this suspension was filtered through a Millipore® membrane with a pore size of 5 μm. The filters were air dried, fixed with methanol, stained with Giemsa's stain and examined by light microscopy for the presence of microfilariae.

Parasite antigen

Adult *B. malayi* worms were purchased from TRS labs, Athens, Georgia. Female worms were freeze dried, ground to powder, dissolved in phosphate-buffered saline (PBS), homogenized and slowly stirred overnight 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.

Table 1. Description of the study population

(Residents from 6 transmigration units were grouped according to length of residence in the new settlement. Life-long residents (LLR) from 2 neighbouring villages were included in the study as a reference group.)

Length of residence	Cases	Children/ Adults*	Males/ females	MF prevalence (%)
⩽ 1 month	17	3/14	14/3	–†
2–4 months	36	12/24	25/11	–†
3 years	67	35/32	39/28	0‡
4 years	54	17/37	30/24	0
5 years	23	8/15	14/9	0
6 years	50	22/28	29/21	12
LLR	133	86/47	92/41	13
Total	380	183/197	243/137	

* Children ⩽ 15 years, adults 16+.
† For logistic reasons blood was collected during daytime in 2 villages, therefore microfilaria counts are lacking in a subset of study participants.
‡ Microfilaria prevalence in the 3-year residents could be determined in 20 individuals only.

Enzyme-linked immunosorbent assay for detection of IgG4

Anti-filarial IgG4 levels were determined in an enzyme-linked immunosorbent assay (ELISA), which has been described elsewhere (Terhell *et al.* 1996, 2000). OD values of patient plasma were converted into arbitrary units (A.U.) by drawing a standard curve of a control plasma from a donor positive for *B. malayi* in Central Sulawesi, Indonesia. A cut-off value for BmA specific IgG4 antibodies was determined by taking the mean IgG4 reactivity (A.U.) plus 3 times standard deviations of 20 healthy Dutch donors at the Blood Bank in Leiden.

Statistical analysis

Statistical analysis was performed in SPSS for Windows version 8.0. For specific IgG4 levels a $\log_{10}$ transformation was used to obtain normally distributed data; in this paper specific IgG4 is the transformed $\log_{10}$ (specific IgG4) level. Levels of specific IgG4 were compared by *t*-test. Chi-square test was used for comparison of proportions; if indicated, Fisher's exact test was used instead. The relationship between anti-filarial IgG4 and length of residence was investigated by calculating Spearman's rank correlation.

The influence of independent variables age, gender, and length of residence on the dependent variable specific IgG4 level (in A.U.) was evaluated by performing multiple linear regression analysis. Various transformations of age were modelled (e.g. linear, natural logarithm, quadratic, square root) before selection of the natural logarithm of age (ln(age)) in the final models, which provided the best fit.

RESULTS

Anti-filarial IgG4 as a function of length of exposure in adults and children

The prevalence of anti-filarial IgG4 in transmigrants and LLR, classified as children (⩽ 15 years) and adults (16 years and older) as a function of length of exposure is shown in Fig. 1A. The levels of this antibody produced by IgG4+ individuals are depicted in Fig. 1B. In transmigrants, there was a clear correlation between levels of anti-filarial IgG4 and length of settlement in the area ($\rho = 0{\cdot}37$, $P < 0{\cdot}001$, $n = 247$). In this group, the prevalence of specific IgG4 (Fig. 1A) increased with duration of exposure from 0% in new arrivals (⩽ 1 month) and 8·3% in recent settlers (2–4 months) up to 63% in 5–6 year residents. Moreover, a clear increase in antibody levels produced by IgG4+ individuals was observed (Fig. 1B). Considering age, LLR adults showed higher levels of anti-filarial IgG4 than children, although this was not statistically significant ($P = 0{\cdot}26$). Several studies have indicated a higher production of anti-filarial IgG4 in adults than in children, and this pattern has often been attributed to the increasing exposure experienced from childhood to adulthood, leading to an accumulation of filarial infection in adults. However, in transmigrants, specific IgG4 prevalence was also higher in adults than in children despite equal length of exposure to filariasis ($P = 0{\cdot}05$ for 3–4 year residents and $P = 0{\cdot}02$ for 5–6 year residents), except for recent settlers (2–4 months, $P = 0{\cdot}32$). In addition, IgG4+ adults were able to produce higher levels of anti-parasite IgG4 than IgG4+ children were. These results indicate that given an equal length of exposure, higher levels of anti-filarial IgG4 were found in adults than in children.

After 5–6 years of exposure, mean IgG4 levels in all transmigrant children (mean IgG4 = 4·56) and adults (mean IgG4 = 5·25) had reached levels that were comparable to native residents (mean $\text{IgG4}_{\text{children}} = 4{\cdot}85$; mean $\text{IgG4}_{\text{adults}} = 5{\cdot}10$; $P = 0{\cdot}13$ and $P = 0{\cdot}52$ respectively). However, anti-filarial IgG4 prevalence in the transmigrant children (47%) remained lower than in LLR children (73%, $P < 0{\cdot}01$). A lower prevalence of anti-filarial IgG4 in transmigrant adults was seen compared with LLR (83%) but this difference was not statistically significant ($P = 0{\cdot}32$). In addition, after 5–6 years of exposure, IgG4+ transmigrants produced higher levels of anti-filarial IgG4 than LLR ($P = 0{\cdot}02$) (Fig. 1B).

Anti-filarial IgG4 as a function of age

The development of anti-filarial IgG4 in different

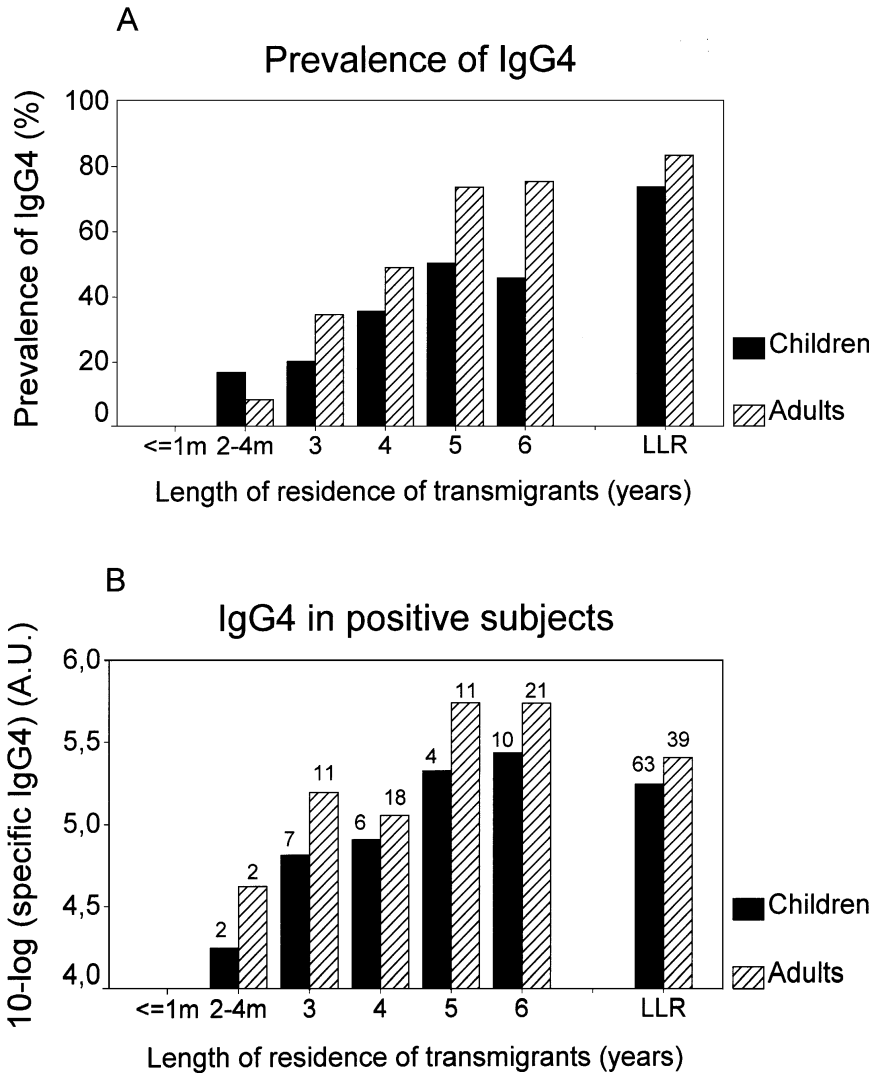

Fig. 1. Prevalence of anti-filarial IgG4 (A) and specific IgG4 levels produced by IgG4+ subjects (B) in transmigrants and life-long residents, classified as children ($\leqslant$ 15 years; ■) and adults (16 years and older; ▨) as a function of length of exposure. In (B) the number of IgG4+ individuals is given above each bar.

age groups was analysed as a function of length of exposure (Fig. 2). A gradual increase in levels of anti-filarial IgG4 was observed with increasing age in the transmigrant population, with varying lengths of residence in the area, as well as in LLR. Despite the higher IgG4 prevalence in children, who were born in the area, compared to children of transmigrants that had settled 5–6 years prior to this study, the shape of the age-curve of mean IgG4 levels in these transmigrants was comparable to the indigenous population. In both groups, anti-parasite IgG4 was maximal in subjects aged 21–40 years, and decreased slightly after the age 40 years.

Multiple linear regression analysis of anti-filarial IgG4

To test statistically for the patterns observed so far, multiple linear regression models were constructed to dissect the influence of length of exposure and host age on the development of anti-filarial IgG4. In the transmigrant population, the linear regression model for specific IgG4 ($F = 17{\cdot}5$, d.f. = (3,244), $P < 0{\cdot}001$) confirmed that development of anti-filarial IgG4 was not only dependent on duration of exposure (in years) ($b = 0{\cdot}20$, $P \leqslant 0{\cdot}001$) but also on age ($b_{\ln(age)} = 0{\cdot}21$, $P = 0{\cdot}03$). Furthermore, there was a strong influence of gender on levels of specific IgG4, with males showing higher anti-filarial IgG4 ($b_{males} = 0{\cdot}27$, $P = 0{\cdot}03$ with females as a reference group). When IgG4+ subjects were considered separately ($n = 92$, $F = 7{\cdot}3$, d.f. = (2,89), $P < 0{\cdot}001$), levels of specific IgG4 were less strongly associated with age ($b_{\ln(age)} = 0{\cdot}24$, $P = 0{\cdot}13$) but increased with length of residence in the area ($b = 0{\cdot}22$, $P = 0{\cdot}001$), and no differences between sexes were observed.

In LLR, regression analysis on specific IgG4 levels ($F = 6{\cdot}5$, d.f. = (2,130), $P = 0{\cdot}002$) confirmed the increase of specific IgG4 with age ($b_{\ln(age)} = 0{\cdot}35$, $P = 0{\cdot}006$), and levels of specific IgG4 were higher in males than in females ($b_{males} = 0{\cdot}33$, $P = 0{\cdot}04$ with females as a reference group). Anti-filarial IgG4

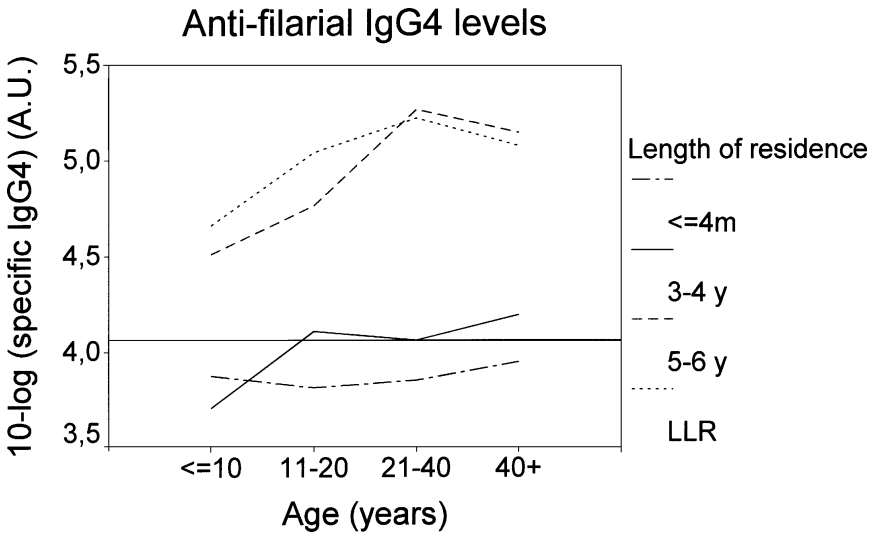

Fig. 2. Anti-filarial IgG4 levels in the transmigrant and indigenous population in different age groups as a function of length of residence in the area. (The straight line represents the cut-off value for specific IgG4 reactivity.)

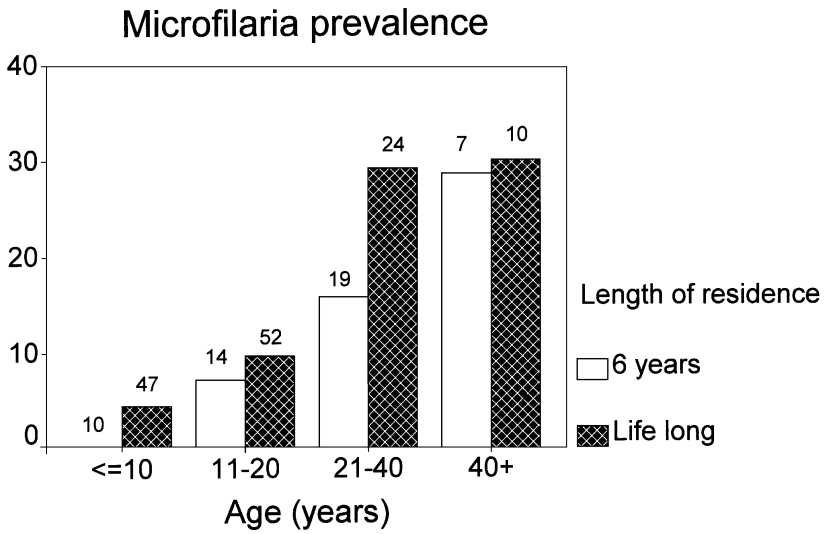

Fig. 3. Microfilaria prevalence (%) in transmigrants, settled in the area for 6 years and in life-long residents in different age groups. The number of subjects in age groups is given above each bar.

levels produced by IgG4+ individuals ($n = 102$, $F = 5{\cdot}5$, d.f. = (1,100), $P = 0{\cdot}02$) were only associated with age ($b_{\ln(age)} = 0{\cdot}26$, $P = 0{\cdot}02$).

Relationship between microfilaria prevalence and age

Microfilariae could first be detected after 6 years of residence in previously unexposed individuals. Fig. 3 shows the prevalence of microfilaraemia in different age groups of transmigrants, after 6 years of settlement, and in LLR. Although children, who were born in the area, developed microfilaraemia at earlier age than transmigrants, the overall prevalence was comparable in both populations (12·8% versus 12·0%, $P = 0{\cdot}89$). Interestingly, the increase in microfilaria prevalence with age, which was observed in the indigenous population, was also evident in the transmigrant population. Within the transmigrant population, prevalence in adults aged 16 years and older (21%, 6/28) was significantly higher than in children ($\leqslant$ 15 years; 0%, 0/22, $P = 0{\cdot}02$), similar to the differences between children (8%, 7/86 and adults (21%, 10/47) observed in LLR ($P = 0{\cdot}03$). These results indicate that adults acquire infection more rapidly than children.

DISCUSSION

The present study was undertaken to investigate the development of microfilaraemia and anti-filarial IgG4, an immunological marker of active filarial infection, in Indonesian transmigrants to determine the role that age *per se* plays in acquisition of *B. malayi* infection. The dissection of the influence of age from that of length of exposure is possible in transmigrant groups because both adults and children have been exposed to filarial infection for identical periods of time since their arrival in the

endemic region. The results showed that specific IgG4 in transmigrants increased with increasing length of residence in the area. Migrants living for 5–6 years in the area had higher levels of specific IgG4 than those residing in the area for 4 years or less. After 6 years of settlement, both anti-filarial IgG4 and microfilaria prevalence in the transmigrant population had reached levels that were comparable to life-long residents. The data show that in this particular area with microfilaria prevalence of 13 %, 5–6 years of exposure is needed for the adult worm burden to reach an equilibrium in the population at a level that allows microfilariae to be detected.

We observed that despite equal length of residence in the *B. malayi* endemic area of all age groups, anti-filarial IgG4 in trasmigrants was consistently lower in children than in adults. In addition, microfilaria burden, detectable in the 6-year-long residents was significantly higher in adults than in children, which is in agreement with previous studies performed in transmigrants on Sulawesi (Tesch, 1937; Partono *et al*. 1972), and confirms that filarial infection establishes more rapidly in adults. Interestingly, the shapes of the age-curves of anti-filarial IgG4 and microfilaria prevalence in transmigrants were similar to the indigenous population. These findings indicate that the development of anti-filarial IgG4 and microfilaraemia is not only determined by length of exposure but depends strongly on intrinsically age-related factors.

Although development of specific IgG4 has not been investigated in a naive population before, 2 previous reports have studied antibody profiles in transmigrants. Piessens, Wadee & Kurniawan (1987) found an increase of IgG directed against soluble *B. malayi* microfilarial antigen between 3 and 14 months after settlement, which is in agreement with the present findings. In another study performed in Indonesia (Kurniawan *et al*. 1990) anti-filarial IgG antibodies failed to correlate to length of exposure (3–6 years), but the number of subjects examined was relatively small.

Several possibilities may account for the observed difference in acquisition of infection between children and adults. First, exposure to infective larvae might be different between the two groups, as a result of either different behavioural patterns or attractiveness for mosquitoes (Takken, 1991). In this area, the main vectors for filarial transmission are *Anopheles barbirostris* and *Mansonia uniformis* (Slee, 1930; Partono *et al*. 1977), which are both indoor-resters and bite mainly at night. Adults may present a greater body-surface, and thereby be subject to greater exposure than children. Secondly, the results could be explained by physiological differences between adults and children, allowing adults to host a greater worm burden than children, resulting in higher microfilaria prevalence and anti-filarial IgG4 levels in older subjects, despite equal exposure. Third, differences in innate immune response between adults and children could underlie the more rapid acquisition of infection by the adult population. The cells and tissues of the immune system are affected, both quantitatively and qualitatively, by maturation and ageing (Wade *et al*. 1988; Horan & Ashcroft, 1997). This is reflected in a greater number of natural killer cells as well as T- and B-lymphocytes in children than in middle-aged people (Sansoni *et al*. 1993). However, the ratios of CD4/CD8 T-cells have been reported to decline from young age onwards (Aldhous *et al*. 1994; Osugi *et al*. 1995). This relative decline of CD4 may be of importance as these cells are essential in animal models for protection against tissue dwelling helminths (Urban, Katona & Finkelman, 1991).

It is interesting to note that studies performed in recent foci for schistosomiasis in Africa revealed that age–intensity profiles of schistosome infection were similar to those seen in communities with life-long exposure to the parasite (Stelma *et al*. 1993; Ouma *et al*. 1998). Therefore it was suggested that age-related phenomena and not prolonged exposure (>10 years) would account for the observed age-stratified immunity. In lymphatic filariasis we found a similar specific IgG4-age curve for life-long residents and transmigrants; an initial increase which levels off after the age of 20 years. If the observed levelling off of the specific IgG4 curve (rather than a continual increase), is related to the acquisition of immunity in older age groups, then the similar patterns of IgG4 in transmigrants and native residents, in analogy with the schistosomiasis studies, would support the observations that under certain conditions it is possible that prolonged and cumulative experience of exposure is not a pre-requisite for acquired immunity. Direct evidence for acquired immunity in humans has come from studies measuring changes in infection levels over time, which showed an increase of parasite burden in children but not in adults (Day *et al*. 1991*b*). This immunity is thought to be mediated by anti-L3 surface antibodies that are present only in older individuals (Day *et al*. 1991*a*). More recently, Michael & Bundy (1998) have developed a mathematical model using data from 19 different published studies which could effectively explain the variations in observed age-prevalence patterns by the occurrence of an exposure-driven acquisition of herd immunity. To reconcile our observations that in a naive population infection establishes more rapidly in adults than in children, with the latter studies showing a higher resistance to incoming L3 in adults, the following needs to be considered. If acquired immunity is dependent on quantitative experience of L3 or adult worms (which could be measured by anti-filarial IgG4 since L3 and adult worms contain cross-reactive epitopes), then an initially faster acquisition of infection in adults may lead to a more rapid build up of immunity to

incoming L3. Therefore, although adults may acquire infection more rapidly due to intrinsic age-related factors they may, after an initial period of exposure, build up immunity more rapidly. To prove this, it would be essential to study transmigrants longitudinally in the same manner as was done by Day *et al*. (1991*b*). It is interesting to note that in studies in transmigrants on Irian Jaya adults developed effective immunity to *Plasmodium falciparum* more quickly than infants (Baird *et al*. 1993).

It was noted that after 5–6 years of exposure IgG4+ transmigrants were able to produce even higher levels of anti-filarial IgG4 than LLR. This could indicate that immunity in transmigrant adults might need a longer time-span to reach its plateau in the population. However, it could also reflect an immunologically naive response, which is uninfluenced by *in utero* exposure to filarial antigens (Weil *et al*. 1983; Lammie *et al*. 1991; Steel *et al*. 1994; King *et al*. 1998).

Many studies in filariasis endemic areas have indicated a higher prevalence of lymphatic filarial infection in males than in females by detection of microfilariae or specific IgG4 (Hitch, Lammie & Eberhard, 1989; Brabin, 1990; Chanteau *et al*. 1995; Haarbrink *et al*. 1995; Michael, Bundy & Grenfell, 1996), and this difference might be hormonally mediated. In the present study prevalence of anti-filarial IgG4 was higher in males than in females, and this was true for both transmigrants and native Sulawesians. This finding indicates that development of infection in previously unexposed individuals is not only regulated by age-related factors, but also by gender, in a similar manner as for the indigenous population.

In conclusion, we have shown that development of anti-filarial IgG4 correlates with duration of exposure in previously unexposed individuals, resulting in development of infection levels comparable to the indigenous population within 5–6 years after settlement in this filarial endemic area. However, infection is established more rapidly in the adult population than in children, which might indicate that either differences in exposure exist between children and adults, or alternatively that age-dependent factors such as physiological or immunological differences regulate this differential gain of filarial infection.

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