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Ecologic and Biologic Determinants of Filarial Antigenemia in Bancroftian Filariasis in Papua New Guinea

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The relationship between filarial antigenemia and lymphatic pathology was investigated in residents of 11 villages in an area of Papua New Guinea where *Wuchereria bancrofti* is endemic. Antigenemia was determined in 1322 persons by means of the Og4C3 antibody capture assay. Prevalence of antigenemia by village ranged from 61.7% to 98.2% and did not vary by sex. Antigen level increased with transmission potential among the 4 villages with measured transmission potential ($r^2 = .945$; $P = .028$). Antigenemia was associated positively with age in villages with the lowest annual transmission potentials (45 and 404 infective larvae/year; $P < .001$), but was distributed evenly across age groups in villages with increased transmission (1485 and 2518 infective larvae/year). These data suggest that children and adults have similar worm burdens in areas of high transmission, whereas worm burdens in areas of lower transmission increase with age. These results may be useful in the design and evaluation of programs aimed at eliminating lymphatic filariasis.

Nearly 1 billion persons in the world are at risk for infection with lymphatic filarial parasites, and ~120 million persons in the tropics are infected with *Wuchereria bancrofti* or *Brugia* species [1]. Although residents of areas of endemicity presumably are exposed to mosquito-borne infective third-stage larvae (L3) throughout life, not all develop patent infection, as defined by the presence of microfilariae in peripheral blood [2, 3], and <10%–20% have clinically overt disease manifestations, such as hydrocele or elephantiasis [1]. Studies of experimental animal models of filariasis and epidemiologic observations of humans suggest that males are at higher risk than females for infection and disease and that this susceptibility may be regulated by both immune and nonimmune pathways [4–6]. The ecologic and biologic variables that contribute to this heterogeneity in infection and lymphatic pathology remain poorly understood.

The number of adult worms established in the lymphatics may be an important variable contributing to the heterogeneity of infection and lymphatic disease in at-risk populations. However, estimation of worm burden is problematic, because direct quantification of lymphatic-dwelling worms in humans is not feasible. The primary means of diagnosing active infection previously relied on the detection of bloodborne microfilariae, the progeny of fecund female worms. This indirect measure did not allow for researchers to determine whether microfilariae-negative status was due to true absence of adult worms, infection with nonfecund worms, infection with single-sex *W. bancrofti,* or asymptomatic infection with microfilarial density below the limits of detection [7]. In addition, subjects could not be classified accurately according to their relative worm burden, because the precise relationship between microfilarial count and adult worm burden was unclear.

The recent development of assays that measure circulating filarial antigens has enabled detection and quantification of infection intensity independent of microfilaremia [3, 7–10]. The Og4C3 filarial antigen was identified originally by a monoclonal antibody raised against the cattle nematode *Onchocerca gibsoni.* In the case of human filariasis, the antibody reacts with antigenic determinants expressed and secreted by *W. bancrofti* adult worms without cross-reactivity to *Brugia* species or other common gastrointestinal or tissue-invasive helminths of humans [7]. Because the level of filarial antigenemia has been shown to decrease after the administration of drugs that kill adult *W. bancrofti* [11, 12], these tests may be useful for quantification of the intensity of infection. In addition, because results of studies that used a 2-site Og4C3 capture ELISA have shown elevated levels of an-

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Informed consent for physical examination and venipuncture was obtained from all adults and guardians or parents of children. The study conformed to the human experimentation guidelines of the US Department of Health and Human Services. The protocols were approved by the Institutional Review Board for Human Studies of the University Hospitals of Cleveland, Case Western Reserve University, and the Medical Research Advisory Committee of the Government of Papua New Guinea.

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tigenemia in many microfilariae-negative residents of areas of endemicity for filariasis [9, 10], the lack of microfilaremia may not accurately reflect the absence of active infection.

We have reported characteristics of microfilarial prevalence and intensity in Papua New Guinea and their relationship to age, sex, and local transmission intensity, as described elsewhere [13–15]. These results can now be compared with results of the use of Og4C3 antigenemia as a reflection of *W. bancrofti* worm burden independent of microfilaremia.

Subjects, Materials, and Methods

Study area and population. Study participants were persons ≥1 year old living in 11 villages in the Dreikikir area of East Sepik Province, Papua New Guinea. Collection of demographic information (date of birth, sex, and village and household of residence) has been described elsewhere [14]. Physical examination for advanced hydroceles in men and grade II–III lymphedema of the leg in both sexes was done according to guidelines outlined by the World Health Organization [16]. The data reported here are based on information collected from 1322 persons during 1993–1994. Systematic control measures against filariasis did not exist at that time, and antifilarial medications were not available. Residents of these villages subsequently participated in a trial to assess the relative efficacy of annual single-dose diethylcarbamazine versus diethylcarbamazine plus ivermectin in interrupting transmission and controlling for disease due to bancroftian filariasis. Results of the first year of the trial have been reported elsewhere [15].

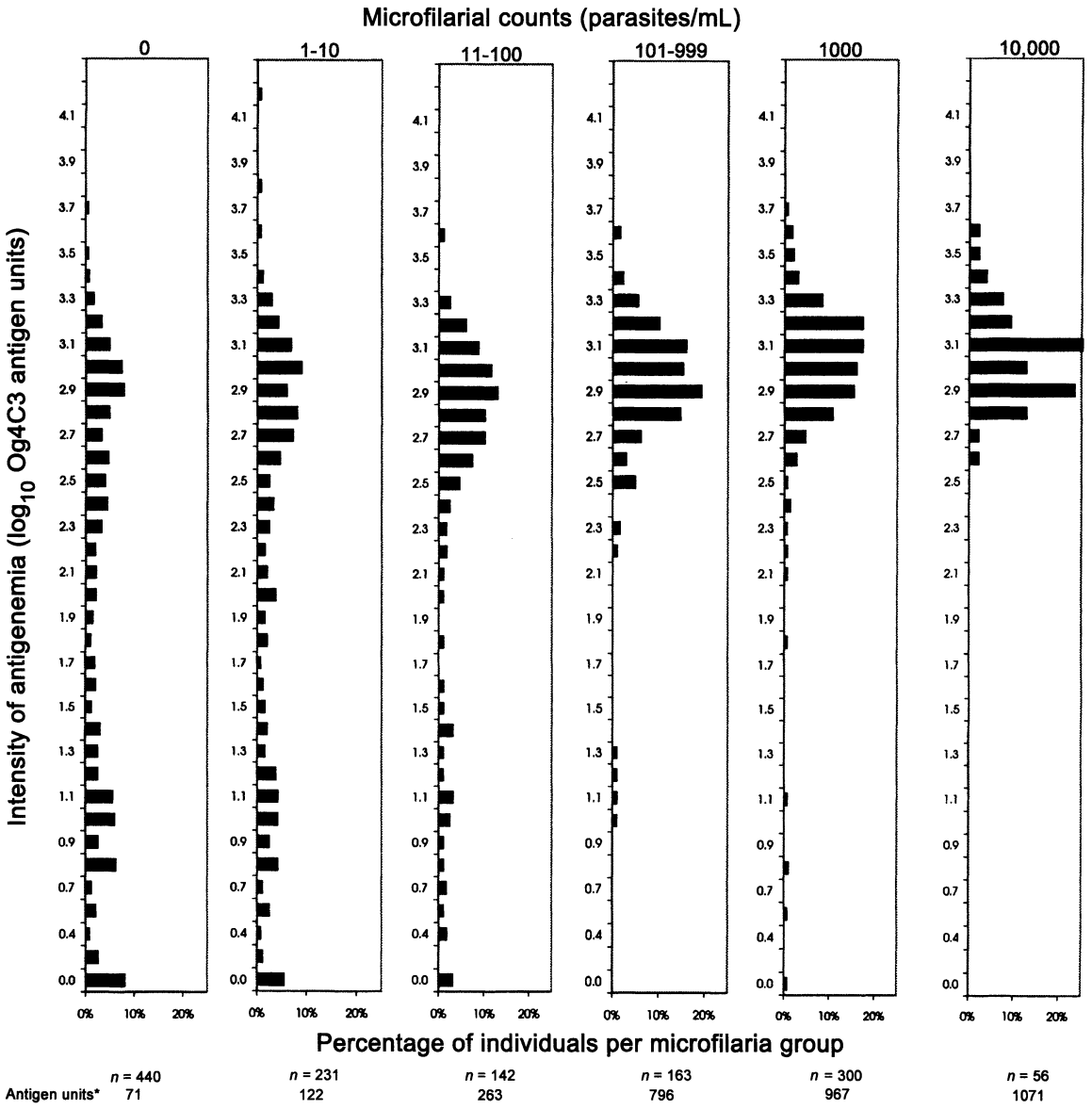

Figure 1. Relationship between Og4C3 antigen and intensity of microfilaremia. Antigen units were obtained from Og4C3 antibody-capture ELISA of plasma samples from 1322 persons. Subjects are grouped according to microfilarial density per milliliter of blood. Variation in antigen units decreased with increasing numbers of microfilariae per milliliter of blood. *Mean antigen units are antilog of mean log.

Table 1. Relationship between age and Og4C3 antigen level among residents of area of endemicity for bancroftian filariasis in Papua New Guinea.

Age group, years	Antigen level[a]			Geometric mean antigen units
	Negative	Low	High	
0–5	16 (59.3)[b]	9 (33.3)	2 (7.4)	26
6–10	71 (45.8)	35 (22.6)	49 (31.6)	67
11–15	68 (37.6)	30 (16.5)	83 (45.9)	111
16–20	40 (27.0)	28 (18.9)	80 (54.0)	189
21–30	57 (18.6)	63 (20.6)	186 (60.8)	303
31–50	31 (9.7)	64 (20.0)	225 (70.3)	499
51–65	15 (9.8)	33 (21.6)	105 (68.6)	465
>65	3 (9.4)	9 (28.1)	20 (62.5)	411

[a] Data are no. (%) of subjects.

[b] Children 0–5 years old were less likely to have high antigenemia than were children 6–10 years old (χ^2, 6.30; $P < .013$). Frequency of low-level antigenemia did not vary significantly by age.

The primary mosquito vector of *W. bancrofti* in East Sepik Province and other areas of Papua New Guinea is *Anopheles punctulatus,* although *Anopheles koliensis* has a limited distribution [17–19]. Villages in the study area are separated geographically from each other by a minimum of 3 km and a maximum of 50 km. The flight distance of the local mosquito vector is estimated to be <2 km [17]. Entomological indices, such as the annual transmission potential, were based on the capture of mosquitoes attempting to feed on adult volunteer residents, as described elsewhere [19, 20]. Individual mosquitoes were dissected, and L3 were identified by morphologic criteria [21, 22]. Entomological data for 4 villages were based on collections done between 6 P.M. and 6 A.M. for 4 nights/month for 1 year.

Parasitologic measurements. Blood was drawn by venipuncture between 10 P.M. and 2 A.M., because microfilaremia has a nocturnal periodicity in this area. Microfilarial counts were determined by light microscopy on 1-mL blood samples subjected to filtration (Nuclepore) [23]. Samples with microfilarial densities ≥1000 microfilariae/mL were categorized by factors of 10, to compensate for the low methodologic precision of counting samples with >1000 microfilariae/mL.

Blood was separated and was stored at −20°C until assays of filarial antigenemia were done. The Og4C3 ELISA was done with boiled plasma diluted 1:4, a negative control, and 7 standard samples with increasing concentrations of *O. gibsoni* antigen, as recommended by the manufacturer (TropBio). Optical density readings of the 7 manufacturer standards were plotted for each microtiter plate and were converted to antigen units, with values ranging from 0 (standard sample 1) to 32,000 (standard sample 7). The results of duplicate samples from study subjects were derived from this standard curve. Subsequently, the optical density readings were placed into 1 of 3 groups of antigenemia: negative, if the optical densities were less than or equal to standard sample 2 (considered to be nonreactors by the manufacturer); low, if the optical density was between that of standards 2 and 4; or high, if the optical density was greater than standard 4.

Statistics. Association among microfilaremia, antigenemia, and dichotomous variables (e.g., female or male sex and presence or absence of lymphedema or hydrocele) were calculated by means of Pearson χ^2 tests. Heterogeneity of antigenemia by age and village were determined with Kruskal-Wallis tests. Correlation coefficients were used to determine the relationship between annual transmission potential and antigenemia. Analysis of variance and the Bonferroni method for multiple comparisons were used to determine the variation in antigenemia among groups of microfilarial densities. $P < .05$ was considered to be significant.

Results

Relationship between antigenemia and intensity of microfilaremia. Figure 1 compares Og4C3 antigenemia among all 1322 subjects grouped by microfilarial density (0, 1–10, 11–100, 101–999, 1000–9999, and ≥10,000 microfilariae/mL). There was a strong positive correlation between mean antigenemia and microfilarial level ($r^2 = .97$; $P < .001$). A Bonferroni test revealed that the 3 groups with the lowest microfilarial density were significantly different from all other density groups. In contrast, the 3 groups with the highest microfilarial density were not significantly different from each other, indicating the convergence of antigenemia at high microfilarial counts. In addition, the coefficient of variation for antigenemia by group was 4 times greater in the 3 groups with the lowest microfilarial density than in the 3 groups with the highest microfilarial density (48.8 and 12.5, respectively). This decrease in antigen range with increasing microfilaremia can be observed in figure 1. Similar relationships were reported by others in studies with smaller sample sizes [9, 10].

Antigen level and age. Og4C3 antigen was detectable in all age groups, including 40% of children between age 0 and 5 years (table 1). The presence of antigen increased until age 30, when the prevalence leveled off at ~90%. Antigenemia increased rapidly with age, with 31.6% of the 6–10-year age group classified as having high antigenemia, a 4-fold increase from the youngest age group. Antigenemia peaked in the 31–50-year age group, as measured by the geometric mean of antigen units and proportion of persons classified as having high antigenemia (70.3% of age group; table 1).

Sex-associated antigen levels. Prevalence and intensity of Og4C3 antigen did not significantly differ by sex. This similarity of antigenemia between sexes was independent of microfilaremic status (table 2) and age (data not shown).

Table 2. Og4C3 antigen level according to sex and microfilaremic status among residents of area of endemicity for bancroftian filariasis in Papua New Guinea.

Sex	Antigen negative	Low antigenemia	High antigenemia
Female			
Total	171 (24.3)	139 (19.7)	394 (56.0)
Microfilaria negative	105 (42.5)	61 (24.7)	81 (32.8)
Male			
Total	130 (21.0)	132 (21.4)	356 (57.6)
Microfilaria negative	82 (42.5)	60 (31.1)	51 (26.4)

NOTE. Data are no. (%) of subjects. There was no significant difference in antigenemia by sex when analyzed without regard to microfilaremic status ($P = .35$) or after stratification for microfilaremic status (Mantel-Haenszel χ^2, 0.28; $P = .260$).

Table 3. Relationship between clinically overt lymphatic pathology and circulating antigen level among residents of area of endemicity for bancroftian filariasis in Papua New Guinea.

Pathology	Antigen negative	Low antigenemia	High antigenemia
Leg lymphedema[a]	11 (22.9)[a]	10 (20.8)	27 (56.2)
Control	95 (12.6)	159 (21.0)	502 (66.4)
Hydrocele[b]	8 (10.8)	13 (17.6)	53 (71.6)
Control	53 (14.2)	76 (20.4)	243 (65.3)

NOTE. Data are no. (%) of subjects. Antigenemia was less likely to be detected in persons with leg lymphedema than in control subjects (χ^2, 4.22; $P <$ .040). There was no significant difference in level of antigenemia and leg lymphedema or of hydrocele, compared with that in control subjects.

[a] Grade II–III leg lymphedema in persons ≥21 years old.

[b] Hydrocele in males ≥16 years old.

Disease manifestations of filariasis and circulating antigen levels. To examine the relationship between antigen level and lymphedema of the extremities, data for subjects ≥21 years old with grade II–III leg lymphedema were analyzed against disease-free persons. Children and adolescents were excluded from the analysis to prevent bias due to their low risk of disease manifestations. Similarly, analysis of hydrocele was limited to males ≥16 years old. Table 3 shows the distribution of antigen levels among persons with leg lymphedema versus disease-free persons and among persons with hydrocele versus disease-free persons. There was no significant difference in level of antigenemia with respect to leg lymphedema or hydrocele. When antigenemia was classified as a dichotomous variable (antigen positive vs. antigen negative), however, persons with leg lymphedema were more likely to be antigen negative than were those without leg lymphedema (χ^2, 4.22; $P < .040$).

Circulating antigen levels and village. Because transmission has been shown to vary by village in this area of Papua New Guinea [14], subjects were grouped according to their village of residence, to determine the relationship between this variable and village-specific heterogeneity of infection. There was a significant difference in antigen levels among the 11 villages examined (χ^2, 220; $P < .001$; table 4). Villages 19 and 13 had more subjects with no or low-level antigenemia than expected from the population as a whole, whereas village 11 had more with high-level antigenemia than expected. These 3 villages accounted for more than half the variation in antigenemia. This heterogeneity could not be accounted for by differences in the age of the persons from whom the samples were collected, because all villages had similar age profiles (data not shown).

Circulating antigen levels and transmission intensity. To gain a better understanding of the factors underlying spatial variation of infection, annual transmission potential was measured in 4 of the 11 study villages (490 persons). The proportion of antigen-negative subjects was greatest in the village where the annual transmission potential was lowest; that is, >50% of 0–10- and 11–20-year-old subjects in the village with a transmission potential of 45 L3/year were antigen negative, compared with <10% in the village where the transmission potential was 2518 L3/year (figure 2, *top*). Figure 2 *(bottom)* describes the relationship between age-specific geometric mean antigen level and transmission intensity. In all villages, <10% of persons ≥41 years old were antigen negative. Mean antigen levels tended to be lowest in 0–10- and 11–20-year-old subjects, except in the village where transmission intensity was 2518 L3/year. In this case, antigenemia exceeded 600 units in all age groups. There was a positive correlation between the village-specific geometric mean level of antigenemia and annual transmission potential ($r^2 = .945$; $P = .014$; slope = .233). Circulating antigen level differed significantly among age groups in the villages with the lowest annual transmission potential (residents of villages with 45 L3/year and 404 L3/year; χ^2, 59.97; $P < .001$). In these villages, antigenemia increased with age. In contrast, antigen level was not significantly different by age in the 2 villages with the highest transmission potential.

Discussion

The risk factors for infection and disease due to *W. bancrofti* have been difficult to characterize because of the complex life cycle of this mosquito-borne helminth and because of the broad range of clinical signs and symptoms attributable to this nematode. Most understanding of these events has been based on the detection of circulating microfilariae as a marker for active infection and overt physical signs of disease, such as lymphedema and hydroceles. Because microfilariae are less likely to be found in the blood of older persons with severe disease [24, 25], it was reasoned that these persons had mounted an effective immune response that eliminated infection but contributed to lymphatic pathology [26–29]. A meta-analysis of published literature also suggested that infection and disease were more prevalent in men than in women [4]. The development of circulating antigen assays that measure worm burden independent of microfilaremia enabled us to reexamine these associations among a large number of persons living in an area of endemicity

Table 4. Distribution of circulating filarial antigen levels in 11 villages in the Dreikikir area of Papua New Guinea.

Village code	Antigen negative	Low antigenemia	High antigenemia
11	2 (2.8)	7 (9.7)	63 (87.5)
1	16 (11.7)	12 (8.8)	109 (79.6)
6	8 (13.3)	8 (13.3)	44 (73.3)
15	34 (19.1)	22 (12.4)	122 (68.5)
20	5 (6.2)	22 (27.5)	53 (66.2)
4	24 (20.5)	16 (13.7)	77 (65.8)
10	27 (18.4)	28 (19.0)	92 (62.6)
12	29 (29.9)	25 (25.8)	43 (44.3)
16	36 (34.6)	24 (23.1)	44 (42.3)
13	59 (39.3)	37 (24.7)	54 (36.0)
19	60 (34.9)	70 (40.7)	42 (24.4)

NOTE. Data are no. (%) of subjects. Two villages were excluded from analysis because of low sample size ($n < 5$). Values are presented in descending order from village with highest to lowest percentage of persons with high circulating antigen levels. Antigenemia varied significantly by village (χ^2, 219.60; $P < .001$).

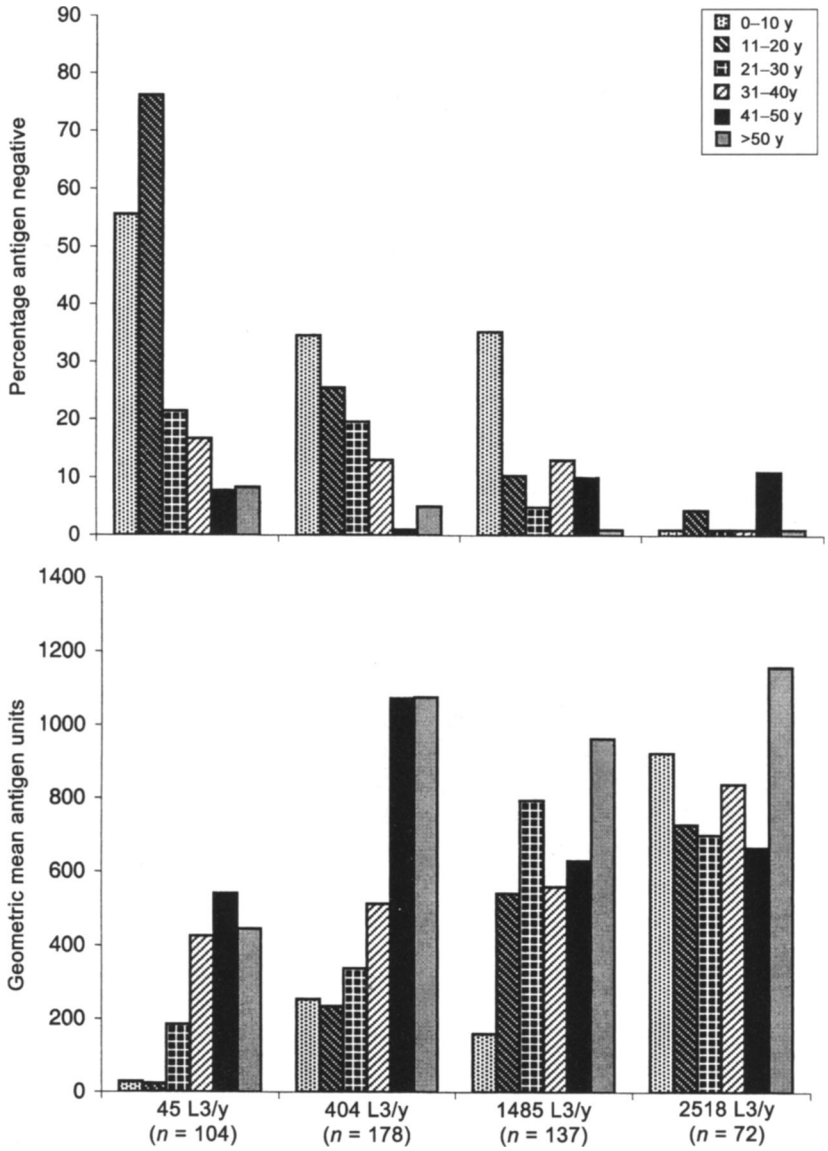

Figure 2. Relationship between antigenemia and annual transmission potential. Percentage antigen negative *(top)* and intensity of antigenemia *(bottom)* in plasma samples from 491 persons grouped by age in 4 villages. Annual transmission potential is represented for each village by the calculated no. of infective L3 exposures per year (L3/y). Intensity of antigenemia was significantly different among age groups in which annual transmission potential was 45 L3/year and 404 L3/year (villages 16 and 15, respectively; χ^2, 59.97; $P < .001$) but not among age groups in which annual transmission potential was 1485 L3/year and 2518 L3/year (villages 1 and 11, respectively).

in Papua New Guinea, where systematic control measures and antifilarial drugs were not previously available.

The data reported here indicate associations that do not support several paradigms implied by earlier studies based on microfilarial status alone. First, there was no evidence of an age-related decrease in infection intensity. On the contrary, the level of antigenemia progressively increased for persons between 0–5 and 21–30 years old. It leveled off thereafter and did not decrease even after age 65 years (table 1). These results suggest that age-related complete or "sterile" immunity to L3, L4, and/or adult-stage *W. bancrofti* is uncommon (e.g., only 9.8% of persons 51–65 years old and 9.4% of persons >65 years old were antigen negative). Similar trends were noted for microfilaremia in this study population [14]. These findings are in contrast with reports that describe a decrease in microfilarial status and intensity with increasing age and disease [30–34]. Differences between results of this study and reports from other areas of endemicity may not simply be related to the use of antigen status versus microfilaremia alone. They may be a result of sample size and selection bias, the extraordinarily high trans-

mission intensity in Papua New Guinea, compared with that in many other areas of the world, or a true biologic difference in hosts, vectors, or parasites in Papua New Guinea relative to other areas of endemicity. Data from our study site were generated from 1322 subjects living in the same communities, whereas many reports are based on observations of fewer persons and/or persons seeking clinical care [32]. In addition, the very high infection pressure in Papua New Guinea may simply overwhelm any differences in susceptibility among males and females. It is not possible to comment substantially on ecologic variation between studies, because transmission intensity has not been intensively quantified or reported for most other areas of endemicity. There is no a priori support for the third possibility, which would require evaluation of genetic polymorphisms as they relate to unknown biologic factors of *W. bancrofti,* vectors, and humans.

No difference in antigenemia according to sex (table 2) or disease status (table 3) was observed among the subjects examined here. Earlier reports of sex differences were based on microfilarial status alone and involved fewer subjects [35, 36]. However, there are well-documented sex-related differences in susceptibility of *Meriones unguiculatus* (Mongolian jirds) to *Brugia* infection [37]. It is not surprising that similar differences do not exist in human bancroftian filariasis, since the host and parasite species are not the same.

An additional objective of the current study was to gain insight into how transmission intensity may influence infection intensity and the propensity to develop clinically overt lymphatic pathology. Understanding such relationships may not only contribute to understanding the strategies the parasite has evolved to maintain fitness (i.e., the success and efficiency of transmission between the vector and host) but also provide practical information for setting priorities to institute control measures (e.g., choosing an area of a country that has the greatest need for distribution of mass chemotherapy). Consistent with our earlier findings of spatial heterogeneity in transmission intensity, microfilaremia, and disease [14], we observed remarkable differences in antigenemia among villages located within 20–30 km of each other (table 4). These differences correlated with transmission intensity; mean antigenemia increased as the annual transmission potential increased ($r^2 = .97$; $P < .001$). Perhaps most striking from this perspective are the results for persons ≤20 years old. Antigenemia among this age group in the 2 villages with the lowest annual transmission potential (45 and 404 L3/year) was <10% of that of age-matched residents of the 2 villages where transmission was 1485 and 2518 L3/year. Indeed, 0–10-year-olds in the village with highest transmission had mean antigen levels greater than those of adults in the lower-transmission sites (figure 2). It is not known whether similar differences in adult worm loads exist among these groups (the Og4C3 antigen is thought primarily to be secreted by adult worms, but its biologic half-life or rate of production by adult worms of varying age and fecundity is not known) [7, 9]. However, since the Og4C3 antigen assay reflects the relative worm burden in a population, the data described here suggest that children living in high-transmission areas should be given high priority in the global program to control bancroftian filariasis [38]. Because the duration of treatment necessary to interrupt transmission of *W. bancrofti* is not known, analysis of antigenemia in this age group may be uniquely informative for control program evaluation.

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