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Reduction of *Wuchereria bancrofti* Adult Worm Circulating Antigen after Annual Treatments of Diethylcarbamazine Combined with Ivermectin in French Polynesia

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Circulating filarial antigen (CFA), determined with Og4C3 ELISA, is a marker of *Wuchereria bancrofti* adult worm infection. The reduction of CFA over 2 years was determined in 185 microfilaremic and 111 amicrofilaremic but CFA$^+$ adults given an annual dose of either diethylcarbamazine (DEC) or ivermectin or the two combined. Reduction of CFA level was good with DEC but weak with ivermectin and followed the same pattern in amicrofilaremic and microfilaremic groups. Combinations and DEC alone had a similar impact on CFA level. CFA clearance was observed in amicrofilaremic but not in microfilaremic persons in all DEC-containing treatments. However, the highest clearance rate was observed in persons treated with DEC at 6 mg/kg combined with ivermectin. Continuous reduction of CFA level after repeated treatments shows that elimination of *W. bancrofti* infection, monitored by CFA clearance, might be achieved within a few years with annual treatments of DEC combined with ivermectin.

At least 108 million people are infected by bancroftian filariasis in Asia, Africa, South America, and the Pacific Islands [1]. Control relies mainly on chemotherapy with diethylcarbamazine (DEC). In French Polynesia, this drug was distributed between 1950 and 1982, leading to a great reduction in prevalence of filariasis. However, in 1993 a control program based on semiannual distribution of DEC at 3 mg/kg was reimplemented due to reemergence of infection. Ivermectin has also been evaluated for its action against lymphatic filariasis [2]. Both drugs, when ingested individually, kill microfilariae (Mf) within 1–4 h and keep the Mf level low until 6 months after treatment [3]. However, the best control was obtained by combining ivermectin and DEC in a single treatment, which maintained the Mf density of <2% of the pretreatment value for at least 1 year [4, 5].

Macrofilaricidal effect of either drug has been a subject of debate due to the lack of diagnostic tools. Indirect evidence has indicated that ivermectin does not kill adult worms but inhibits reproduction [2], while DEC is partially macrofilaricidal [6]. Visual observation of adult worms by ultrasound techniques has confirmed these findings [7, 8].

During the last decade, two monoclonal antibody ELISAs have been developed to detect adult worm circulating filarial antigens (CFA) in humans [9, 10]. Both assays can be used on blood collected during the day. They have shown that a large proportion of amicrofilaremic subjects are CFA$^+$ and therefore suspected of having adult worms [11–13]. However, most of the trials to monitor the macrofilaricidal effect of drugs have been done in microfilaremic persons [4, 11], except a single study reporting the effect of DEC in Mf$^-$ CFA$^+$ persons [14]. In the present study, we assessed the efficacy of annual treatments of DEC, ivermectin, or a combination of both drugs on CFA levels in persons harboring only adult worms or adults and Mf, using the Og4C3 antigen ELISA.

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Informed consent was obtained from all subjects according to protocols approved by the Ethical Committee of Institut Malardé and French Polynesia Health Authorities.

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Materials and Methods

Patients. A large-scale chemotherapy study on Tahaa Island, French Polynesia, evaluated the effect of annual treatments of ivermectin and DEC, given singly or in combination, on Mf prevalence [5]. On this island of ~4000 inhabitants, the DEC distribution was stopped in 1982; a decade later, transmission of filariasis was occurring according to entomologic indices and to the high prevalence of antifilarial antibodies (78%) (data not shown). In February 1994 and February 1995, 1881 adult inhabitants (mean age, 41 years; range, 20–94; 54% men and 46% women) received a single annual treatment with one of the following doses, resulting in 4 treatment groups: DEC at 6 mg/kg (DEC6), ivermectin at 400 μg/kg (ivermectin), and ivermectin at 400 μg/kg plus DEC at 6 mg/kg (ivermectin + DEC6) or at 3 mg/kg (ivermectin + DEC3). Venous blood was collected during the day (*Wuchereria bancrofti* is subperiodically diurnal in Polynesia) before the first (month 0) and second (month 12) treatments and 1 year after the second treatment (month 24). Microfilaremia was determined by membrane filtration of 1 mL of blood, and sera were separated by centrifugation and stored at −30°C until antigen assay.

Antigen assays. Levels of CFA in sera were determined with the Og4C3 antigen ELISA [10] (Trop Ag *W. bancrofti* assay; JCU Tropical Biotechnology, Townsville, Australia). The results were expressed in arbitrary antigen units per milliliter using *Onchocerca gibsoni* antigen as standard (cutoff = 100 U/mL). Antigen assay was performed on pretreatment sera (month 0) from 46–47 Mf^+ subjects per group randomly selected. Sera from Mf^- persons from the 4 groups were tested at random with antigen assay until ~30 CFA^+ persons per group were identified. Efficacy of treatments to reduce CFA levels were then determined by using sera collected at months 0, 12, and 24 from Mf^+ persons (46–47/group) and Mf^- CFA^+ persons (27–28/group).

Data analysis. The association between CFA values and microfilaremia were examined by Pearson's correlation coefficient. The effects of treatments on Mf and CFA levels were assessed by measuring residual geometric means of levels at months 12 and 24 compared with those before treatment. The variations of effects between the treatment groups were compared by F test ($P = .05$) and Newmann-Keuls post hoc comparisons. The percentages of persons cleared of Mf or CFA were compared between groups with the χ^2 test.

Results

Infection status prior to treatment. Before treatment (month 0), the Mf prevalence in the adult population of Tahaa ($n = 1881$) was 22%, with microfilaremia ranging from 1 to >10,000 Mf/mL [5]. Of 187 Mf^+ persons (1–6816 Mf/mL) selected for antigen assay, 185 were CFA^+ (98.9%). The 2 CFA^- persons had only 1 and 4 Mf/mL. There was a positive correlation between CFA level and microfilaremia ($r = .59$, $P < .05$). The CFA assay, done on 354 Mf^- subjects, was positive for 111 (31%). By extrapolating those data, ~46% of the adult population of the island was CFA^+ (22% Mf^+ and 24% Mf^-).

Effects of treatments on Mf and CFA. In Mf^+ subjects (table 1), the geometric mean microfilaremia ranged between 454 and 645 Mf/mL, and the geometric mean CFA level ranged from 3170 to 5126 U before treatments. The 4 groups were satisfactorily matched for initial microfilaremia ($P = .13$) and antigenemia ($P = .08$). Combinations of ivermectin and DEC provided the best microfilaricidal effect, with a reduction of 46%–50% in the number of Mf carriers and a residual microfilaremia of 2% of subjects at month 24, although this was not significantly different from results with DEC treatment. In contrast, microfilaremia at month 24 was significantly less reduced with ivermectin treatment than with the 3 other treatments ($P = .03$). In addition, the reduction in number of Mf carriers was significantly greater at month 24 in the 2 combination groups than in the ivermectin group ($P < .002$), and there was a trend for a greater reduction in Mf in the DEC group than in the ivermectin group (not significant).

The highest reduction of CFA levels in Mf^+ persons was observed in treatments that included DEC, either alone or combined with ivermectin, but no significant difference was observed between different treatments that included DEC. A moderate effect of ivermectin on antigenemia was observed; this was significantly lower than effects of treatments that included DEC ($P = .02$). However, none of the Mf^+ persons were cleared of CFA, even at month 24, except 1 individual (2.2%) treated with ivermectin + DEC3.

In Mf^- CFA^+ subjects (table 2), the geometric mean CFA level before treatment ranged from 487 to 963 U and was not significantly different between groups. CFA levels were 4.2–6.8 times lower than CFA levels in Mf^+ subjects. Ivermectin showed a low efficacy in clearing CFA, as the residual CFA level at month 24 was still 82% of baseline (month 0) and no Mf^- subject was cleared of CFA. In contrast, the 3 treatments that included DEC, alone or combined with ivermectin, greatly reduced the CFA level by month 24 to <19% of baseline, but there were no significant differences between the 3 treatment groups. In groups treated with a regimen that included DEC, 7%–12% of subjects had clearance of CFA at month 12, but there were no significant differences between the 3 groups. At month 24, CFA clearance in these groups ranged from 17%–44% and was significantly higher in the ivermectin + DEC6 group than in the DEC6 or ivermectin + DEC3 groups ($P < .03$). The reductions in number of CFA carriers in the DEC6 and ivermectin + DEC3 groups were not significantly different. Reductions of CFA levels were significantly greater in Mf^- than in Mf^+ subjects ($P = .01$) when results from the 4 treatment groups were combined.

Discussion

An annual single-dose treatment of DEC combined with ivermectin has a better efficacy on early and long-term reduction of *W. bancrofti* Mf prevalence and density than either drug given individually [3–5]. Elimination of filariasis requires, in addition, killing of adult worms, which act as parasite reservoirs. Indirect evidence has shown that the Og4C3 antigen ELISA can detect adult worm infection [12]. Additional evi-

Table 1. Evolution of intensity and prevalence of microfilaremia and circulating filarial antigen in microfilaremic persons after filaricidal drug treatments.

	DEC6 (n = 46)	IVR (n = 46)	DEC6 + IVR (n = 47)	DEC3 + IVR (n = 46)
Microfilariae				
Pretreatment microfilarial intensity (Mf/mL)				
Geometric mean	645	563	487	454
Range	5–4301	2–4709	6–6816	2–3706
% residual microfilaremia				
Pretreatment	100	100	100	100
Month 12	9.4	13.5	2.9	3.5
Month 24	2.8	10.5	1.8	2.0
% reduction in Mf carriers				
Month 12	11.1	10.7	17.8	28.5
Month 24	35.7	17.8	46.4	50.0
Circulating filarial antigen				
Pretreatment antigen level (Og4C3 antigen U)				
Geometric mean	5045	3537	3170	5126
Range	1032–20,416	744–15,428	244–9800	1132–12,008
% residual antigen level				
Pretreatment	100	100	100	100
Month 12	55.5	70.0	60.0	52.3
Month 24	37.6	63.3	41.0	27.0
% reduction in antigen carriers				
Month 12	0	0	0	2.2
Month 24	0	0	0	2.2

NOTE. DEC6 = diethylcarbamazine at 6 mg/kg, IVR = ivermectin at 400 μg/kg, DEC6 + IVR = diethylcarbamazine at 6 mg/kg and ivermectin at 400 μg/kg, DEC3 + IVR = diethylcarbamazine at 3 mg/kg and ivermectin at 400 μg/kg.

dence was provided in the present study, in which DEC (in contrast to ivermectin) had a dramatic effect on reduction and clearance of CFA in Mf^- subjects. Moreover, evolution of CFA levels according to treatment was not linked with the presence of Mf, as CFA levels followed the same pattern in Mf^+ and Mf^- persons. Nevertheless, biochemistry experiments should help to clearly identify and localize the target antigen(s) of the Og4C3 monoclonal antibody.

The sensitivity of the CFA assay for detecting *W. bancrofti* infection was excellent, since it identified 99% of Mf^+ persons. This sensitivity was somewhat higher than reported in a previous study, in which the detection limit was 50 Mf/mL [12]. In our study, CFA was also detected in one-third of Mf^- subjects. These data indicated that the prevalence of adult worm burden was about twice the Mf prevalence. This confirms other data obtained in French Polynesia, using the same antigen assay, on Raiatea Island [12] and the Marquisas Islands (Nicolas L, et al., unpublished data) and in other areas with endemic filariasis [13]. It means that there were approximately as many cryptically infected persons as microfilaremic persons. Although the role of the cryptically infected in the epidemiology of the infection remains undefined, they could act as parasite reservoirs. With a goal of elimination of filariasis, it was therefore important to determine the impact of filaricidal drugs in those persons as well.

Ivermectin had a poor effect on CFA level. However, in Mf^- subjects, some had a significant reduction of CFA level after ivermectin treatment. This might reflect individual variations in susceptibility to ivermectin between parasites or drug response between patients. From this study, the best results in Mf and CFA reduction were obtained with treatments that included DEC. Combined DEC6 and ivermectin, compared with DEC6 alone, had a significant effect on the clearance of CFA, but the impact on reduction of antigen level was not different between the 3 treatments that included DEC. This confirms data from a previous study on Moorea Island with a small number of subjects (n = 19) [4]; a continuous decrease in CFA levels after repeated treatments was observed in all groups receiving treatment that included DEC. Another study on Moorea Island of 19 Mf^+ persons treated for 3 years with an annual dose of ivermectin + DEC6 showed a residual CFA level of 40%, 32%, and 15% after one, two, and three treatments, respectively (Nicolas L, et al., unpublished data). Despite a continuous decrease in CFA level, only 1 individual (2.2%) was cleared of CFA by month 24 among Mf^+ subjects. This patient initially had 238 Mf/mL and 1828 CFA U/mL. However, CFA clearance was reached in a significant proportion of Mf^- subjects, probably because their initial CFA level was 4–7 times lower than that in Mf^+ persons.

Table 2. Evolution of intensity and prevalence of circulating filarial antigen in amicrofilaremic persons after filaricidal drug treatments.

	DEC6 (n = 28)	IVR (n = 28)	DEC6 + IVR (n = 27)	DEC3 + IVR (n = 28)
Antigen level (Og4C3 antigen U)				
Geometric mean	736	829	488	963
Range	194–3859	135–3141	123–1478	187–3912
% residual antigen level				
Pretreatment	100	100	100	100
Month 12	22.3	85.6	33.8	43.0
Month 24	16.4	82.0	8.4	18.8
% reduction in antigen carriers				
Month 12	21.4	0	19.2	7.1
Month 24	25.0	0	44.4	17.8

NOTE. DEC6 = diethylcarbamazine at 6 mg/kg, IVR = ivermectin at 400 μg/kg, DEC6 + IVR = diethylcarbamazine at 6 mg/kg and ivermectin at 400 μg/kg, DEC3 + IVR = diethylcarbamazine at 3 mg/kg and ivermectin at 400 μg/kg.

Therefore, clearance of *W. bancrofti* infection (Mf and adult worms) with annual doses of DEC, alone or combined with ivermectin, is likely to be achieved within a few years. Clearance of infection should take longer in highly infected persons. Annual strategy was evaluated in Polynesia for practical aspects of drug distribution. However, maximal reduction of CFA level was achieved ~6 months after treatment [4]; therefore, more frequent treatments, if feasible, could be considered for those subjects to speed up CFA clearance.

In attempts to eliminate *W. bancrofti* infection, CFA assay is a promising tool for monitoring infection, although Mf detection remains the single method to determine whether an individual can transmit the parasite. CFA assay defines better than microfilaremia whether a person is infected and avoids night blood sampling, which is necessary in most areas with endemic filariasis for Mf detection. CFA clearance can be considered a marker of parasite clearance, but complementary investigation by ultrasonography of CFA-cleared subjects should be helpful. Monitoring reduction of the CFA level requires ELISA, which is a quantitative assay, but CFA clearance could be also determined with individual finger-prick tests, which are under development. Finally, CFA monitoring can be completed by screening pooled mosquito populations by polymerase chain reaction [15].

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