Clearance of Circulating Filarial Antigen as a Measure of the Macrofilaricidal Activity of Diethylcarbamazine in *Wuchereria bancrofti* Infection

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Small doses of diethylcarbamazine (DEC) clear microfilariae (MF) from the blood of *Wuchereria bancrofti*–infected persons, but the dose and regimen required to kill adult worms is not clearly defined. A prospective study was undertaken to examine the macrofilaricidal effect of DEC and the ability of an assay for circulating filarial antigen (CFA) to define the effect. Twenty-five MF-positive subjects and 7 MF-negative but CFA-positive subjects were treated with DEC and followed for 18 months. Of the 25 MF-positive patients, 24 cleared MF, and 22 of 26 CFA-positive subjects cleared CFA. A significantly greater decrease in antifilarial IgG4 was seen in patients who cleared CFA than in those who did not. The complete clearance of CFA observed after therapy with DEC indicates that assessment of CFA clearance is a useful means for detecting macrofilaricidal effects of antifilarial chemotherapy.

Lymphatic filariasis caused by the filarial parasites *Wuchereria bancrofti, Brugia malayi,* and *Brugia timori* occurs in many areas of the tropics, with an estimated 100 million people infected and 900 million at risk for acquiring infection [1]. The most prominent clinical manifestations of infection—lymphedema, elephantiasis, and hydrocele—result from chronic lymphatic damage induced by the parasite and the host's response to it and from recurrent bacterial infections in tissues with impaired lymphatic function.

While the drug diethylcarbamazine (DEC) has been available for treatment of lymphatic filariasis for almost 50 years, the dosage required to cure infected persons has never been clearly defined [2]. Even a small dose of the drug effectively clears microfilariae (MF) from the blood of infected persons [3, 4], but the dose required to kill the adult parasite (the macrofilaricidal dose) has been essentially impossible to define because of the inability to detect living adult worms within the human host (at least until the very recent application of ultrasound techniques [5]). Thus, macrofilaricidal effects could only be inferred by documenting the failure of microfilaremia to recur in treated persons.

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The recent development of monoclonal antibody–based assays capable of detecting parasite antigen in the peripheral blood of *W. bancrofti*–infected persons [6, 7] has been a major advance in the diagnosis of filarial infections. The assays detect patent infection, obviate collection of blood samples when MF are present in the peripheral circulation (at night in many areas) [8], and permit diagnosis of cryptic (amicrofilaremic) infections and assessment of therapeutic success by monitoring the disappearance of circulating filarial antigen (CFA) from the blood [7, 9, 10]. This approach, which is currently being used to assess the macrofilaricidal effects of new drugs for the treatment of filariasis (e.g., ivermectin), may also lead to improved dosage regimens for older drugs, such as DEC. Previous studies have documented a general decrease in CFA levels in patients treated with DEC or ivermectin [7, 9–12]; however, to date, there are no published reports of complete clearance of antigen from the circulation of infected persons after treatment.

To define more clearly the macrofilaricidal effect of DEC and to observe clearance of CFA from the blood of infected persons, we prospectively evaluated a group of *W. bancrofti*–infected patients after therapy with either of two regimens of DEC; we assessed changes in microfilaremia, CFA, and IgG- and IgG4-specific antifilarial antibody levels. The IgG4 antibody subclass was of particular interest because studies indicate that elevated levels of these antifilarial antibodies reflect ongoing active infection in humans [13–15]; thus, a decline in antifilarial IgG4 levels might predict a successful (i.e., macrofilaricidal) outcome of therapy.

Materials and Methods

Study population. In June 1992, all 627 residents of the *W. bancrofti*–endemic island of Mauke (Cook Islands), were evaluated for filarial infection by clinical history, physical examination, blood filtration for microfilaremia, and assay of blood for CFA.

All persons with positive tests for MF or CFA (or both) were asked to participate in the study. Exclusion criteria included serious underlying medical conditions, pregnancy, or intolerance to DEC. In addition, 12 persons with no evidence of infection were included as controls.

Parasitologic data and serum samples from a subgroup of subjects who had been studied 17 years earlier [16, 17] were used for comparison with current data. Mass DEC chemotherapy had been undertaken 5 years before the study, so the prevalence and intensity of infection were considerably less than in 1975 (see Discussion).

Clinical and laboratory evaluation. A complete history was obtained from all subjects, and all participants were given a physical examination and clinical laboratory evaluations (hematology, clinical chemistry, and urinalysis). All women had negative urine pregnancy tests before receiving DEC.

Treatment protocol. Subjects were randomly assigned to one of two treatment regimens. One group received 8 mg/kg of DEC each evening for 7 days (1-week group). The other group received the same dosage regimen, but it was repeated each month for 5 additional months (1-week/month × 6). Drug was administered under direct observation by hospital staff to ensure compliance. At 15 months, all subjects received a further week of therapy (8 mg/kg/day) as part of an island-wide campaign to eradicate infection. Drug side effects were evaluated by passive surveillance of study subjects.

Specimen collection. Blood specimens were obtained from all patients for evaluation of microfilaremia, CFA, and antifilarial antibodies at study entry, monthly for 6 months after commencement of the study, and at 18 months (for infected but not control subjects). Since microfilaremia is subperiodic in Mauke, blood specimens always were collected between 8:00 AM and noon [16]. Serum and plasma samples were separated from blood samples and frozen for later study.

Assessment of MF. Microfilarial density (MF per milliliter) was determined by filtering 1 mL of blood through a 3-μm pore–size filter (Nuclepore, Pleasanton, CA). Most pretreatment values represent the mean MF count of 2 samples taken over the month before treatment. At the 1- to 6-month time points, 1 mL of blood from each subject was preserved in 9 mL of formalin-teepol, as previously described [18], and filtered. Fresh blood samples were filtered again at 18 months.

Antifilarial antibody determination. Antifilarial IgG and IgG4 antibody levels to microfilarial and adult-stage *B. malayi* antigens were determined at each time point by standardized ELISA, as described previously [17]. All samples from individual subjects were run together in duplicate at two dilutions. Antigens used were saline extracts of the adult and microfilarial stages of the closely related filarial parasite *B. malayi* [17].

Detection of CFA. Soluble *W. bancrofti* antigen was detected in heat-treated human sera or plasma (final dilution 1:2) by a monoclonal antibody–based antigen capture ELISA [6]. Serum samples from all time points for each subject were tested on a single ELISA plate. The testing laboratory was blinded to the treatment regimens for subjects. Antigen levels (nanogram equivalents per milliliter) were obtained by interpolation of mean optical density (OD) values obtained with duplicate serum samples on a standard curve, which was obtained with a *Dirofilaria immitis* adult worm antigen standard that was run in duplicate on each ELISA plate. Samples with OD values not on the linear portion of the standard curve were diluted and retested. Samples with ODs >3 SD above the mean of a panel of MF-negative control sera from 10 North Americans with no history of residence in a filariasis-endemic area were considered positive (cutoff OD = 0.2). The lower limit of detection for this assay is ~3 ng/mL (protein content of the antigen standard), which corresponds to an antigen concentration of 6 ng/mL in human serum. Subjects with positive filarial antigen tests in pretreatment sera and negative tests after treatment were considered to have cleared CFA.

Statistical analysis. Grouped data were compared using the Mann-Whitney *U* test. The relative effectiveness of the two drug regimens was compared by construction of Kaplan-Meier plots for clearance of parasite antigen, with differences assessed by the χ^2 test. The nonparametric Spearman's rank test was used to evaluate correlations.

Results

Infection and CFA status before treatment. Of 627 subjects tested, 34 (6%) were either microfilaremic (27 subjects) or amicrofilaremic but CFA positive (7 subjects). One CFA-positive subject with 56 MF/mL was pregnant and, therefore, not treated with DEC until delivery of her child; she was, however, followed in a fashion identical to the other subjects and remained microfilaremic and CFA positive for the duration of the study. Two other subjects (1 from each group) died of unrelated causes before the end of the study. They were 72 and 80 years old and had counts of 100 and 8 MF/mL, respectively, and were positive for CFA. Data for these 2 patients were excluded from further analysis.

The 2 treatment groups were satisfactorily matched for age, sex, MF counts, and CFA status and levels (table 1). MF counts ranged from 0 to 1029 MF/mL, with a median of 32 MF/mL among those with microfilaremia. CFA levels ranged from 0 to 58 ng Eq/mL, with a median of 10 ng Eq/mL among those positive. Six (22%) of the 27 microfilaremic subjects were CFA negative before treatment; they had a median of 2 MF/mL (range, 1–80). There was a statistically significant correlation between the initial levels of CFA and MF ($r = .42$, Spearman's rank test; $P = .02$).

Clearance of MF after treatment. Most patients, regardless of treatment group, cleared microfilaremia: 23 of 24 evaluable subjects had complete clearance and remained MF negative for the duration of the study. The subject who did not clear MF after DEC initially had 769 MF/mL, the highest count in the 1-week group; by 18 months, her MF count had decreased to 14 mL.

Clearance of CFA after treatment. Of the 26 CFA-positive subjects, 22 cleared their antigenemia by 18 months (figure 1). Of the 4 subjects who did not clear CFA, 3 were in the 1-week/month × 6 treatment arm (pretreatment counts of 155, 9, and 0 MF/mL, respectively), and 1 was in the 1-week treatment arm (pretreatment MF count of 0). The 2 MF-positive subjects who did not clear CFA both cleared MF. Changes in CFA levels in the 2 treatment groups followed a similar pattern, with the geometric mean level decreasing to 50% and 10% of the initial level by 1 and 4 months, respectively. Although there

Table 1. Clinical and demographic data for 44 subjects in study of macrofilaricidal effect of DEC.

Characteristic	Treatment group		
	1-week (n = 14)	6-month (n = 18)	Control (n = 12)
Sex, men/women	10/4	8/10	9/3
Age, years, median (range)	47 (6–74)	37 (9–80)	44 (3–58)
Microfilarial count, mf/mL, median (range)	21 (0–769)	34 (0–1029)	0
Circulating filarial antigen level, ng Eq/mL (median range)	11 (0–58)	10 (0–35)	0
Microfilaremic			
Circulating antigen–positive	10	8	0
Circulating antigen–negative	2	4	0
Amicrofilaremic, circulating antigen–positive	2	6	0

NOTE. Data are no. unless otherwise stated. mf = microfilariae.

was a trend toward earlier clearance of CFA in the 1-week/month × 6 treatment arm (figure 1), there was no statistically significant difference between the 2 groups in the rate of antigen clearance. Five of the 7 subjects who were amicrofilaremic but CFA positive at study entry cleared CFA within 3 months of initial treatment. Two of these 7 became MF positive (6 and 1 MF/mL, respectively) at 1 month; all 7 were MF negative for the rest of the study.

Various CFA clearance patterns were seen: some patients had rapid clearance but others required more time (figure 2A). Among those not clearing CFA, an initial decrease in CFA level was seen but not sustained, and levels remained elevated for the duration of the study (figure 2B). Of interest, subjects who did not clear CFA had significantly higher levels of circulating antigens at study entry than those who did clear CFA (P = .02). Furthermore, there was a direct correlation between the initial level of circulating antigen and the time required to clear antigenemia (r = .61, Spearman's rank test; P = .003).

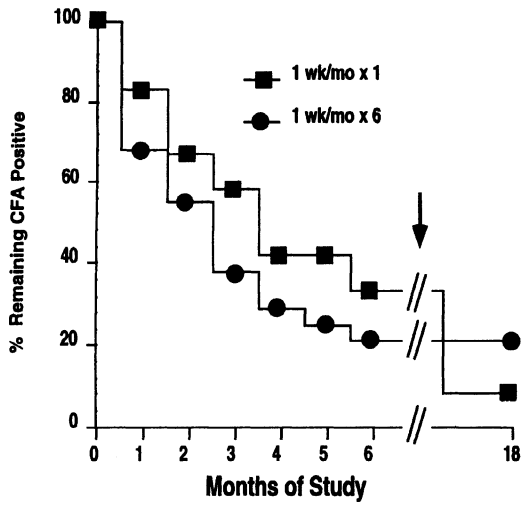


Figure 1. Percentage of patients remaining CFA positive after treatment with DEC. Treatment groups represented by squares (1 week) and circles (1 week monthly × 6 months). Arrow indicates additional 1-week DEC treatment at 15 months.

Antifilarial antibody levels after treatment. Pretreatment serum IgG and IgG4 antibody levels to filarial antigen were elevated in all subjects. Among CFA-positive subjects, there was an almost universal rise in antifilarial antibody levels at 1 month (figure 3). Antibody levels generally declined from 1 to 6 months and reached their lowest levels at 18 months, with 25 of 26 CFA-positive persons having lower IgG levels and 24 having lower IgG4 levels. At 18 months, 14 subjects had antifilarial IgG antibody levels that had returned to levels seen in uninfected controls, while only 2 subjects had IgG4 levels that had decreased to the normal range. There were no differences between the 2 treatment groups when changes in antifilarial antibody levels were compared (data not shown). Also, after DEC therapy, patients who were MF negative but CFA positive had changes in antifilarial antibody levels similar to those in patients who were CFA and MF positive (figure 3). There was, however, a significantly greater decline in antifilarial IgG4 from baseline to 18 months in the subjects who cleared CFA than in those who did not (geometric mean levels at pretreatment and 18 months, respectively: 33,500 and 7496 arbitrary U/mL in the group that cleared CFA vs. 14,826 and 8020 in those who did not; P = .04); this difference was not seen for IgG.

Further analysis of the changes in antibody responses showed that subjects with MF but no detectable CFA had changes in antifilarial antibody levels after DEC therapy similar to those in patients who were MF and CFA positive (data not shown). There was also no significant change in levels of antifilarial IgG and IgG4 from baseline to 6 months among the 12 control subjects tested, and essentially identical results were obtained when microfilarial antigen was used instead of adult-stage antigen to assess antibody responses (data not shown).

Discussion

Previous studies have documented a decline in CFA after therapy with DEC [9–12]. However, our observation of complete clearance of CFA in 22 (85%) of 26 treated subjects is especially important because it confirms the value of assessing

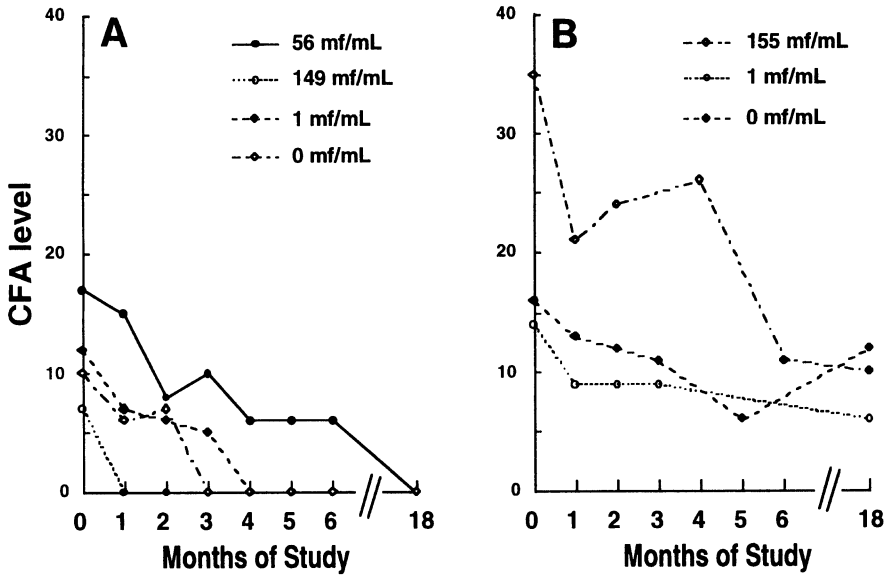
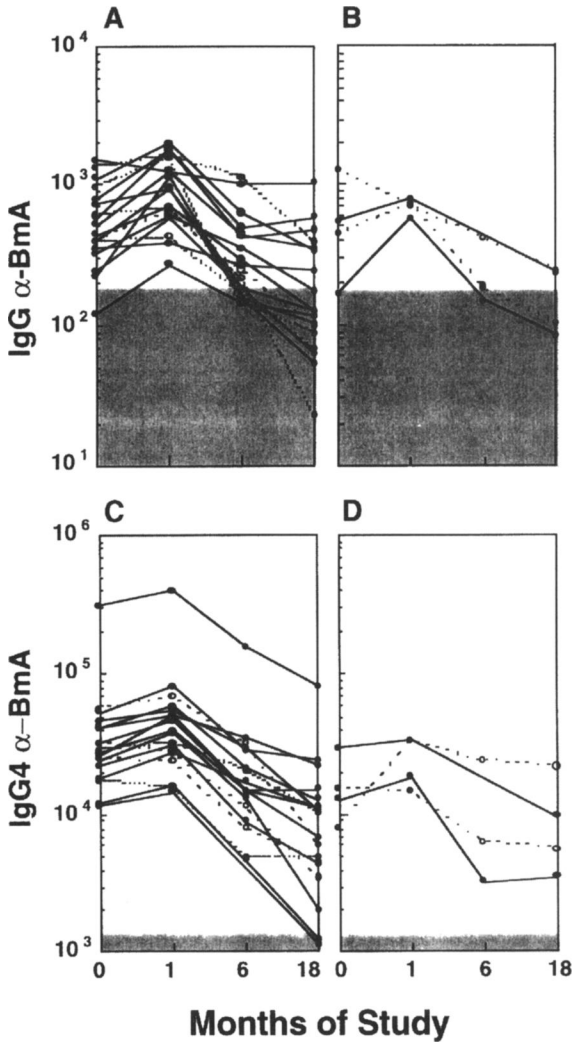


Figure 2. Clearance patterns in 4 of 22 patients who cleared CFA (**A**) and in 3 of 4 patients who remained CFA positive (**B**). Pretreatment blood microfilaria (mf) counts are indicated by symbols. CFA levels are nanogram equivalents per milliliter.



clearance of CFA as a tool for evaluating responses to therapy in individual patients, for comparing the macrofilaricidal effects of different drugs or treatment regimens in clinical trials, and for monitoring large-scale control programs.

This study has a number of unique aspects providing opportunity to document complete clearance of CFA in successfully treated persons. First, the effective interruption of transmission of infection through case finding and selective treatment of all infected persons followed by the community-wide treatment of all islanders eliminated the possibility of reinfection during the follow-up period. Second, the extended period of follow-up was also important because 4 subjects had delayed clearance of parasite antigenemia (between 6 and 18 months). Third, it is likely that the relatively low intensity of infection in this population significantly improved the ability to observe the effectiveness of the DEC therapy and, thus, the complete clearance of CFA.

The relatively low intensity of infection is evidenced by the lower levels of MF in infected subjects compared with levels in the same population studied 17 years earlier (median counts, 269 vs. 32 MF/mL in 1974 and 1992, respectively) [16] and by the lower current pretreatment levels of CFA in 2 patients positive in 1975 and 1992 (247 and 207 vs. 12 and 18 ng Eq/mL, respectively). Furthermore, other studies done with persons from French Polynesia, where subperiodic *W. bancrofti* is also endemic, have shown levels of CFA significantly higher than those observed in the present study [19].

Since microfilaremia and circulating antigenemia generally coexist [20], it was of particular interest to examine the in-

Figure 3. Antifilarial antibody levels (arbitrary U/mL) of IgG (**A** and **B**) and IgG4 (**C** and **D**) in subjects who did (**A** and **C**) or did not (**B** and **D**) clear CFA after DEC therapy. ● and solid lines, microfilarial and CFA-positive subjects; ○ and dashed lines, MF-negative but CFA-positive subjects. BmA, *Brugia malayi* adult-stage antigen.

stances of discordance observed in the present study. First, there were 7 patients who were CFA positive but MF negative. Generally, it has been presumed that such persons have cryptic infections—infections with living adult worms but no microfilaremia; indeed, evidence from animal models supports this explanation [21, 22], but conclusive proof of the infection status of such patients is difficult to obtain. However, our current observation that 5 of 7 such persons cleared CFA after DEC therapy provides considerable support for the hypothesis that these patients did indeed have cryptic infections.

The second discordant group was the 6 subjects negative for circulating antigen despite the presence of microfilaremia. Except for 1 subject with 80 MF/mL, MF levels in this group were low (1–3 MF/mL). Each subject had tests negative for CFA on multiple pretreatment and all posttreatment samples of serum and plasma. There is little doubt that these persons were infected, as their blood samples were repeatedly positive for MF, all had a positive polymerase chain reaction–based assay for *W. bancrofti* DNA in their blood (unpublished data), and all showed clearance of MF and decreases in antifilarial antibodies after DEC treatment. The false-negative assay results from these 6 subjects reduced the sensitivity of the CFA assay to 78%, a figure significantly lower than previously reported [6]. The most likely explanation for this discrepancy is the very much lower intensity of infection in this population compared with that in other areas where CFA assays have been evaluated [7, 8, 10, 20].

The decline in antifilarial IgG4 antibody levels that accompanied the clearance of CFA is consistent with earlier observations demonstrating an association between antifilarial antibodies of the IgG4 subclass and active infection [13–15]. Previous studies have documented a transient rise in antifilarial antibody levels after treatment, peaking at 1 month and declining thereafter [9, 23]. The present study demonstrated that there was a significantly greater decline in IgG4 antibody levels in subjects who cleared their infections than in those who did not. This difference was not seen when total antifilarial IgG levels were compared. While such changes in antifilarial antibody levels are of interest, IgG and IgG4 levels returned to levels seen in uninfected controls in only 14 and 2, respectively, of 26 subjects. Furthermore, since there was overlap in decline of IgG4 antibody level between those who cleared antigen and those who did not, assessment of antibody levels after treatment appears to be less useful for determining therapeutic success than does monitoring of CFA levels.

When these assays were used to evaluate the relative effectiveness of the two treatment regimens, a trend toward more rapid CFA clearance was seen in the group treated with multiple courses of DEC, but the difference did not reach statistical significance. The equivalent efficacy of the two regimens was surprising in light of previous findings that showed that while short courses of therapy effectively clear microfilaremia [3, 4], more prolonged therapy is generally necessary to cure infection [2]. The likely explanation for this finding is the low intensity of infection in our study population and the low level of transmission of infection on the island. Mass chemotherapy administered to all islanders 5 years earlier may have reduced parasite viability so that any further exposure to DEC would have had an enhanced effect on remaining parasites.

With the observation that CFA assays can determine if patients are cured of bancroftian filariasis, it should be possible to design and carry out studies to define the optimal antifilarial chemotherapy regimen for currently available agents, including DEC, ivermectin, and albendazole, and for agents being developed. Furthermore, and perhaps even more important, the widespread application of the CFA assay appears to offer an ideal means of monitoring large-scale filariasis control programs, which are being contemplated for the eventual elimination of this infection.

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