Kinetics of Circulating Human IgG4 after Diethylcarbamazine and Ivermectin Treatment of Bancroftian Filariasis

C. Njeri Wamae, Jacquelin M. Roberts, Mark L. Eberhard, and Patrick J. Lammie

Department of Parasitology, Tulane University, New Orleans, Louisiana; Kenya Medical Research Institute, Centre for Microbiology Research, Nairobi; Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia

Patent filarial infections are associated with elevated levels of parasite-specific IgG4. This study investigated the shifts of filarial-specific human IgG and IgG4 antibodies after diethylcarbamazine and ivermectin treatment of bancroftian filariasis. Thirty adult Haitians were treated first with a 1-mg clearing dose of ivermectin and then with either one or two 200-μg/kg doses of ivermectin or with 12 daily 6-mg/kg doses of diethylcarbamazine. Posttreatment levels of antifilarial IgG4 were dependent on both treatment group and time of follow-up. IgG4 increased markedly to a maximum by day 30 in all treatment groups and then began to decrease; the greatest decrease was among diethylcarbamazine-treated patients. Posttreatment microfilaremia was inversely correlated with the decrease in IgG4; thus, shifts in IgG4 were associated with treatment response for all groups. Antifilarial IgG levels were not correlated with drug treatment and did not change to the same degree as did IgG4 responses.

Lymphatic filarial diseases, caused by the tissue-dwelling nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, are a major cause of morbidity in the tropics. Global estimates indicate that >100 million people are infected with these parasites and the number of infected individuals is increasing [1]. The recognition of correlations between the clinical spectrum of filariasis and the expression of antifilarial immunoreactivity has stimulated efforts to characterize the nature of this relationship [2]. Although filarial parasites elicit antibodies of all major immunoglobulin subclasses, chronic antigenic stimulation, a characteristic of infections with circulating microfilariae, may be responsible for the prominence of IgG4 in antifilarial responses [3–5]. Thus, persistent filarial infection may stimulate and maintain abnormally high IgG4 production. Whether the elevated IgG4 is caused by specific parasite products or by the mode of the host's immune response to filarial infection is not clear, but in either case, clearance of parasites would be expected to eliminate the cause of IgG4 stimulation. In the present study, long-term follow-up of filariasis patients provided an opportunity to assess the relationship between parasite clearance and the kinetics of antibody responses after drug intervention.

Received 18 November 1991; revised 3 February 1992.

Informed consent was obtained from all study participants, and the study was conducted after review and approval by the Human Subjects Committee of the Centers for Disease Control.

Use of trade names is for identification only and does not imply endorsement by the US Public Health Service or Department of Health and Human Services.

Financial support: National Institutes of Health (Y02-00005); UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (to C.N.W.).

Reprints or correspondence: Dr. Patrick J. Lammie, Parasitic Diseases Branch/F13, Centers for Disease Control, 1600 Clifton Rd., Atlanta, GA 30333.

The Journal of Infectious Diseases 1992;165:1158–60

0022-1899/92/6506-0032$01.00

Materials and Methods

Selection and treatment of patients. A detailed protocol for the selection and treatment of the patients has been previously reported [6]. Briefly, 30 Haitian patients with *W. bancrofti* microfilaremia were given a 1-mg oral dose of ivermectin (phase 1) as a microfilaria-clearing dose. Subsequently, participants were randomized into three groups: Group 1 received diethylcarbamazine (6 mg/kg/day for 12 days), group 2 received a single 200-μg/kg dose of ivermectin, and group 3 was given 400 μg/kg ivermectin (200 μg/kg/day for 2 consecutive days). Venous blood samples were collected before and after treatment, and the separated sera were frozen for later analysis. Sera used in this study were collected from January 1989 through January 1991.

Antigen. Soluble *Brugia pahangi* adult worm antigens were prepared as reported previously [7].

IgG assay. The assay to determine serum levels of *B. pahangi*–specific IgG was done as described before [8]. Immulon 2 microtiter plates (Dynatech Laboratories, Chantilly, VA) were sensitized with *B. pahangi* antigen (1 μg/ml) in bicarbonate buffer overnight at 4°C and blocked with PBS–0.3% Tween 20 (Sigma, St. Louis) for 1 h at room temperature. Sera (20 μl) were diluted 1:50 in PBS–0.05% Tween 20, and 25 μl was added to the sensitized plates in triplicate. Biotinylated monoclonal anti-human IgG (Zymed Laboratories, San Francisco), streptavidin–alkaline phosphatase conjugate (Bethesda Research Laboratories, Gaithersburg, MD), and substrate were added in sequence with intermediate PBS–0.05% Tween 20 washings. The reaction was stopped after 10 min with 0.1 *M* EDTA. The optical density of the reaction product was read (405 nm) using a UVmax ELISA plate reader (Molecular Devices, Palo Alto, CA). Dilutions of pooled sera from microfilaremic donors were used as a

standard and arbitrarily assigned a value of 10,000 units of antibody/ml.

IgG4 isotype assay. The assay to determine IgG4 isotype–specific antifilarial antibody levels was done as above except biotinylated monoclonal anti-human IgG4 was substituted for anti-IgG.

Statistical analysis. Antibody levels were analyzed using analysis of variance for repeated measures. Differences in percentage changes in IgG4 levels were tested by the Wilcoxon signed ranks test. The relationship between changes in IgG4 and residual microfilaremia was analyzed and described using linear regression analysis. All tests were two-tailed.

Results

On the basis of levels of detectable residual microfilaremia, both diethylcarbamazine and ivermectin were effective microfilaricides. At both 1- and 2-year follow-up, all treatment groups had a <10% geometric mean return of microfilaremia to pretreatment levels (figure 1) [6, 9]. To determine whether there were serologic correlates of decreased microfilaremia, filaria-specific antibodies were measured by ELISA. Significant pretreatment IgG4 responses were detected in 27 of 30 patients (range, 267–51,950 units/ml). Pretreatment levels of IgG4 were not related to age, sex, or microfilaremia level.

Changes in the levels of antifilarial IgG4 for the 27 patients with significant IgG4 responses before treatment are shown for each treatment regimen in figure 1. Expressed as a percentage of the pretreatment response, comparable shifts were observed for individual patients despite the broad range

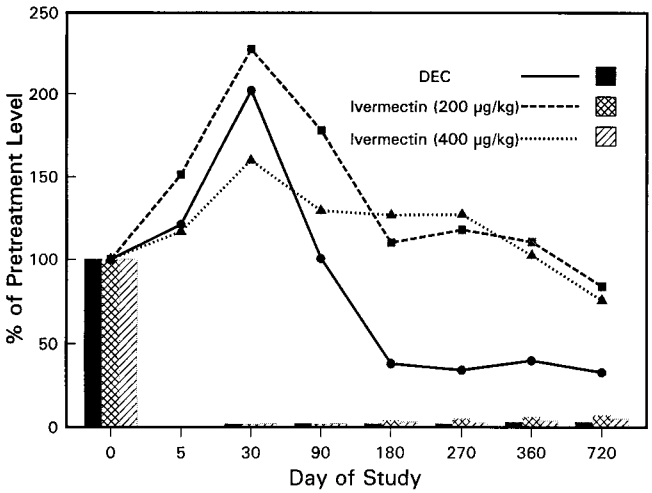

Figure 1. Changes in microfilaremia and antifilarial IgG4 after treatment of *Wuchereria bancrofti* with diethylcarbamazine (DEC) or ivermectin. Geometric mean microfilaremias for three drug regimens at each time point are indicated by bars. For antibody responses, data were normalized for each group by relating IgG4 values for individual patients on indicated day to pretreatment response for that patient. Geometric mean IgG4 responses are shown.

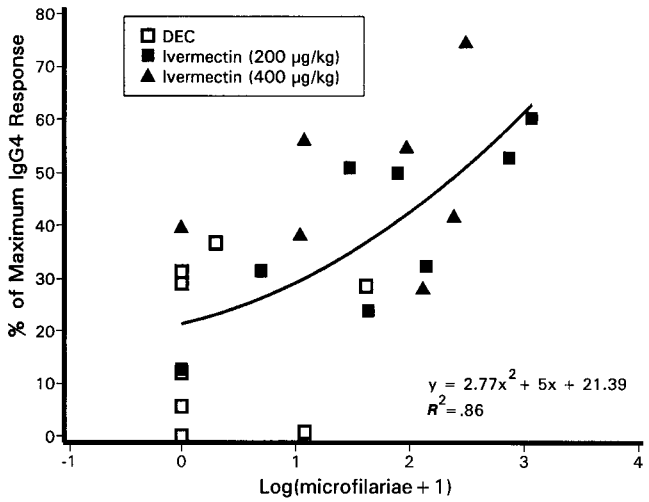

Figure 2. Correlations between antifilarial IgG4 and microfilarial levels after treatment with diethylcarbamazine (DEC) or ivermectin. IgG4 response of individual patients (symbols) on day 720 was expressed as percentage of maximum IgG4 response for that patient and plotted against log of number of microfilariae/ml (+1) on day 720.

of pretreatment IgG4 values (data not shown). IgG4 levels increased in all three treatment groups to a maximum by day 30. A significant drop in IgG4 from day 30 was apparent by day 180, when data for all three groups were pooled ($P < .001$; data not shown). A closer examination of the data by treatment group showed that the greatest decrease was seen in the diethylcarbamazine-treated group. IgG4 responses among diethylcarbamazine-treated patients were significantly lower than pretreatment values during days 180–720 after treatment ($P < .03$ for all time points). IgG4 responses among ivermectin-treated patients were different from baseline values at 2-year follow-up for patients treated with 400 µg/kg, but the change was of borderline statistical significance ($P = .10$).

The data were further analyzed to determine the relationship between changes in IgG4 and treatment response. Figure 2 shows the relationship between residual IgG4 levels and the resurgence of microfilaremia at day 720. There is a strong positive correlation between microfilaremia and IgG4 levels, expressed as a percentage of the maximum IgG4 response. The data are well characterized by the quadratic equation $y = 2.77x^2 + 5x + 21.59$ ($R^2 = .86$).

Changes in IgG responses were not related to either treatment group or treatment response. In addition, although the IgG responses appeared to follow the same trends as those of IgG4, the drops were less marked (data not shown).

Discussion

Microfilaricidal treatment with either diethylcarbamazine or ivermectin was associated with gradual decreases in antifi-

larial IgG and IgG4. Changes in IgG and IgG4 were qualitatively similar and followed shifts in circulating antigen previously reported [10]. Mean antigen levels on day 5 were slightly higher than pretreatment values in all treatment groups. Increases in IgG4 at day 30 may be triggered by the elevations in antigen titer at day 5, increases that have been attributed to dying or damaged parasites after treatment [10]. Clearance of parasites and antigen would be expected to remove the cause of stimulation. In fact, decreased IgG4 levels followed the drop in titers of circulating antigen. A similar phenomenon has been observed in schistosomiasis, another IgG4-inducing helminthiasis, after praziquantel chemotherapy [11]. Despite the evident relationship between reductions in circulating antigen and antifilarial IgG4, only a moderate correlation existed between the percentage change in antigen and IgG4 in individual patients at 1 year (r = .45, Spearman rank correlation coefficient; data not shown).

Differences in the kinetics of IgG4 between the different treatment groups provide further evidence that diethylcarbamazine and ivermectin differ in their filaricidal efficacy. Diethylcarbamazine treatment leads to a greater reduction in microfilaremia, circulating antigen, and antifilarial IgG4 (figure 1) [6, 9, 10]. These observations suggest that diethylcarbamazine has a macrofilaricidal effect. Clinical reactions compatible with adult worm killing also were restricted to diethylcarbamazine-treated patients [6].

The relationship between IgG4 responses in treated patients who remained amicrofilaremic at 2-year follow-up versus those who had residual microfilaremia also implies that a proportion of the IgG4 response is directed against adult worms. The curve describing the relationship between posttreatment IgG4 levels and microfilaremia does not intersect zero. In a recent study of patients infected with *Onchocerca volvulus*, antifilarial antibodies of the IgG4 subclass were suggested to be an indicator of low-level infections or those characterized only by the presence of adult worms [12]. Our results are also consistent with this conclusion.

Studies of bancroftian filariasis in Papua New Guinea further indicated that antifilarial IgG4 levels may be a useful seroepidemiologic assay [4]. However, IgG4 monitoring is of limited use as a tool for diagnosis of patent infection. For reasons that are not clear, 10% of patients in this study did not have a significant IgG4 response before treatment and failed to develop a response during the 2-year follow up. In addition, we have observed that antifilarial IgG4 responses increase with age in amicrofilaremic children [8] (unpublished data). It is not clear whether this increase is related to prepatent or occult infection or to continuous exposure to infective larvae. Longitudinal studies of amicrofilaremic children are required to determine the prognostic value of antifilarial IgG4 responses.

In summary, IgG4 responses in treated patients change as a function of antigen level, parasitemia, and adult worm load. On the basis of these changes, diethylcarbamazine has a more pronounced macrofilaricidal effect than ivermectin.

Acknowledgments

We thank David McNeeley of the Ste. Croix Hospital and the other members of the filariasis team for their assistance with this study. Parasites used to prepare antigen were provided by the National Institute of Allergy and Infectious Diseases, US-Japan Cooperative Medical Science Program.

References

1. World Health Organization. Lymphatic filariasis. Fourth Report of the WHO Expert Committee on Filariasis. WHO Tech Rep Ser **1984**;702:3–12.
2. Ottesen EA. Immunologic aspects of lymphatic filariasis and onchocerciasis in man. Trans R Soc Trop Med Hyg **1984**;78(suppl):9–18.
3. Lal RB, Ottesen EA. Enhanced diagnostic specificity in human filariasis by IgG4 antibody assessment. J Infect Dis **1988**;158:1034–7.
4. Kwan-Lim GE, Forsyth KP, Maizels RM. Filarial-specific IgG4 response correlates with active infection. J Immunol **1990**;145:4298–305.
5. Aalberse RC, van der Gaag R, van Leeuwem J. Serologic aspects of IgG4 antibodies. 1. Prolonged immunization results in an IgG4-restricted response. J Immunol **1983**;130:722–6.
6. Richards FO Jr, Eberhard ML, Bryan RT, et al. Comparison of high dose ivermectin and diethylcarbamazine for activity against bancroftian filariasis in Haiti. Am J Trop Med Hyg **1991**;44:3–10.
7. Lammie PJ, Leiva LE, Ruff AJ, Eberhard ML, Lowrie RC Jr, Katz SP. Bancroftian filariasis in Haiti: preliminary characterization of the immunological responsiveness of microfilaremic individuals. Am J Trop Med Hyg **1988**;38:125–9.
8. Hitch WL, Hightower AW, Eberhard ML, Lammie PJ. Analysis of isotype specific antifilarial antibody levels in a Haitian pediatric population. Am J Trop Med Hyg **1991**;44:161–7.
9. Eberhard ML, Hightower AW, McNeeley DF, Lammie PJ. Long-term suppression of microfilaremia following ivermectin treatment. Trans R Soc Trop Med Hyg (in press).
10. Weil GJ, Lammie PJ, Richards FO Jr, Eberhard ML. Changes in circulating parasite antigen levels after diethylcarbamazine and ivermectin treatment of bancroftian filariasis. J Infect Dis **1991**;164:814–6.
11. Jassim A, Hassan K, Catty D. Antibody isotypes in human schistosomiasis mansoni. Parasite Immunol **1987**;9:627–9.
12. Steel C, Lujan-Trangay A, Gonzalez-Peralta C, Zea-Flores G, Nutman TB. Immunologic responses to repeated ivermectin treatment in patients with onchocerciasis. J Infect Dis **1991**;164:581–7.