Og4C3 Circulating Antigen: A Marker of Infection and Adult Worm Burden in Wuchereria bancrofti Filariasis

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Og4C3 Circulating Antigen: A Marker of Infection and Adult Worm Burden in *Wuchereria bancrofti* Filariasis

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Og4C3 circulating filarial antigen was detected in the sera of 94.5% (259/274) of microfilaremic patients, 32% (239/751) of persons with presumption of filariasis, and 23% (11/48) of chronic filariasis patients. The antigen level was correlated with the microfilariae (Mf) density and patient age ($P < .01$). It remained stable in patients treated with microfilaricidal drugs. Og4C3 antigen, undetectable in Mf culture media, was demonstrated to be a rare somatic Mf antigen. It appears to be an excreted or secreted antigen from adult filaria. It could be used as a marker of infection and an indicator of adult worm burden.

The diagnosis of *Wuchereria bancrofti* infection using direct parasitologic methods is hampered by the existence of a large proportion of amicrofilaremic but infected patients. The current filarial antibody detection tests remain unable to distinguish active and past infections, and there is no direct way to measure the parasitic burden. A simple antigen-capture ELISA using a monoclonal antibody (Og4C3) directed against a nonphosphorylcholine and heat-stable antigen of *Onchocerca gibsoni* has been described [1]. We will show, by indirect methods, that Og4C3 circulating antigen is a *W. bancrofti* adult worm antigen.

Materials and Methods

Patients from French Polynesia. Three groups of untreated patients were tested for antigenemia and microfilaremia (determined by membrane filtration of 1 mL of venous blood): 274 asymptomatic microfilariae (Mf) carriers, 751 persons living in endemic villages and positive for antifilarial IgG and IgG4 but amicrofilaremic (Mf-negative) (no clinical examination was assessed), and 48 patients with chronic filariasis, either Mf-positive or -negative.

The effects of microfilaricidal drugs on antigenemia and Mf density were assessed in Mf-positive patients treated with diethylcarbamazine, ivermectin, or both [2] (unpublished data). The immediate effects (1, 2, 4, and 8 h) were evaluated in 3 groups of 5 patients each treated with 6 mg/kg diethylcarbamazine, 400 μg/kg ivermectin, or diethylcarbamazine plus ivermectin. Urine samples were also collected from the last group of patients. The effects of a single dose of 400 μg/kg ivermectin during the first week and then at 6 and 12 months after treatment were assessed, respectively, in 2 groups: 11 male Mf carriers and 30 (12 female and 18 male) Mf carriers.

The effect of repeated semestral doses of ivermectin was studied in patients selected from a community trial in which all inhabitants were given three semestral doses of 100 μg/kg ivermectin, followed by a single dose of 400 μg/kg ivermectin [2]. Every 6 months, the antigenemia and the microfilaremia were monitored in 53 selected persons: 10 Mf carriers, 10 persons Mf-negative throughout the study but antigen-positive before the trial (month 0), and 33 persons Mf-negative at month 0 but found Mf-positive during the study (at month 6, 12, or 18).

Antigen and antibody detection. The Og4C3 circulating antigen test was done by capture sandwich ELISA [1] according to the manufacturer's recommendations (JCU Tropical Biotechnology, Townsville, Australia). The sera were boiled with an EDTA solution, in the kit, to release Og4C3 antigen possibly trapped in immune complexes. After centrifugation, the supernatants were used for antigen testing. The results were expressed in arbitrary antigen units per milliliter using *O. gibsoni* antigen as standard (cutoff = 100 units/mL). The specificity determined for sera of 50 persons from a nonendemic country was 100%.

The anti-*Brugia malayi* IgG and IgG4 ELISA tests have been described [3, 4]. Soluble adult *B. malayi* antigen extract was provided by E. Ottesen (NIH, Bethesda, MD). Specific IgG results were expressed in arbitrary units per milliliter according to a high-titered standard reference pool of sera (cutoff = 600 units/mL); specific IgG4 results were expressed in micrograms per milliliter according to a calibrated standard pool of sera (cutoff = 4 μg/mL).

Anti-sheath antibodies were detected by a conventional indirect immunofluorescence assay (IFA) using native purified *W. bancrofti* Mf as antigen [5] (cutoff titer = 10).

Excreted or secreted (ES) and somatic antigens from W. bancrofti Mf. The Mf purified from infected human blood were cultivated at 37°C for 8 days in RPMI 1640 supplemented with 30% normal human serum (1000 Mf/mL). ES Og4C3 antigen was sought in the supernatant. An extract of Mf-somatic antigens was obtained by sonication of 50,000 Mf in 1 mL of 0.01 *M* PBS (pH 7).

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Table 1. Antigen results in three groups of subjects living in villages endemic for *W. bancrofti*.

Group tested (*n*)	No. (%) antigen-positive	Antigen geometric titer (units/mL)
Asymptomatic, Mf-positive (274)	259 (94.5)*	1676
Mf-negative, specific IgG- and IgG4-positive (751)	239 (32)	740†
Chronic filariasis patients (48‡)	11‡ (23)	446

NOTE. Mf, microfilariae.
* Mf density higher than antigen-negative group (geometric mean 343 vs. 2 Mf/mL, $P < .01$, Student's *t* test).
† Lower than Mf carriers ($P < .01$, Student's *t* test).
‡ Includes 3 Mf-positive patients.

Results

Untreated subjects. Antigen data from the 3 groups of persons tested are summarized in table 1. Antigen was detectable in 259 (94.5%) of 274 Mf carriers tested (Mf density range, $1–10^4$ Mf/mL; geometric mean [GM] = 265 Mf/mL). All 214 patients with an Mf density of >50 Mf/mL were antigen-positive. The antigen level was significantly correlated with Mf density ($r = .59$, $P < .01$, multilinear regression model with log-transformed data). Of the 751 Mf-negative persons living in endemic villages and positive for anti-*B. malayi* IgG and IgG4 (presumption of filariasis), 239 (32%) were antigen-positive. The antigen level was significantly and positively correlated with age ($P < .01$, after adjustment of microfilaremia using a multilinear regression model), but it was not related to sex. Of the Mf-negative antigen-positive patients, 85% (57/67 tested) were positive for anti-sheath antibodies (GM titer = 19).

Of the 48 patients with chronic clinical symptoms of filariasis, 11 were antigen-positive (23%), and 3 of these were Mf-positive. All of the remaining 37 antigen-negative patients were Mf-negative.

Effects of microfilaricidal drugs on Og4C3 antigenemia in Mf-positive patients. Despite the immediate and sharp reduction (90%–100%) of Mf density during the first 8 h after treatment, antigenemia remained at the pretreatment level whatever the treatment given (diethylcarbamazine, ivermectin, or both). It was also remarkably stable during the first week in patients treated with 400 μg/kg ivermectin: 91%, 95%, and 93% of residual antigenemia at 1, 2, and 7 days after treatment, respectively, whereas the residual Mf density was <1%. Og4C3 antigen in urine was detectable neither before nor for 8 h after treatment with diethylcarbamazine plus ivermectin despite the high circulating antigen level (1444 units/mL) of the corresponding sera.

Six and 12 months after a single dose of 400 μg/kg ivermectin, the residual GM antigenemia percentage declined slightly but not significantly (figure 1A), and there was no significant difference between female and male subjects (79% and 89%). In contrast, the microfilaremia was still reduced and was significantly lower in female than in male subjects (5% vs. 25%, $P < .01$, Student's *t* test).

After three semestral doses of 100 μg/kg ivermectin plus a single dose of 400 μg/kg ivermectin, the residual GM percentage of Mf in 10 Mf carriers was 23%, 26%, 15%, and 5%, whereas the residual GM antigenemia was 100%, 100%, 96%, and 81%, respectively, at month 6, 12, 18, and 24 (figure 1B). For the 10 Mf-negative subjects, it was, respectively, 120%, 130%, 114%, and 87%.

Of the 33 subjects first classified Mf-negative but found to be Mf-positive by later samples, 24 (73%; Mf density, 1–612 Mf/mL) were antigen-positive at month 0 (GM titer = 2472 units/mL). Their residual GM antigenemia was 85% at month 24. The other 9 persons (Mf density, 1 or 2 Mf/mL) remained antigen-negative throughout the study.

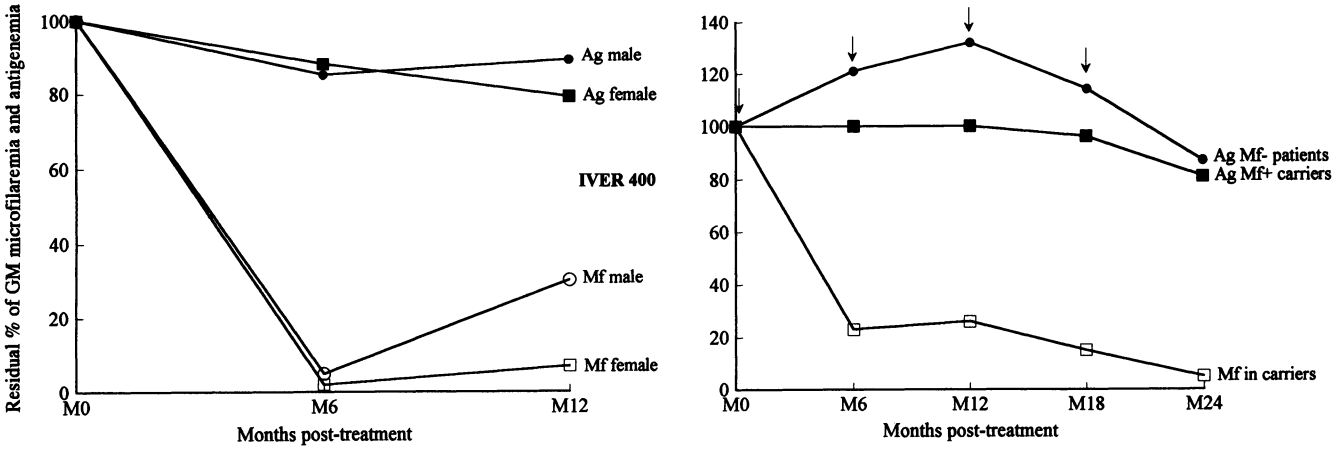

Figure 1. **A**, Long-term effect of single dose of 400 μg/kg ivermectin on antigenemia in 12 female and 18 male microfilariae (Mf) carriers. **B**, Effect of 4 repeated semestrial single doses of ivermectin (3× 100 μg/kg ivermectin and 1× 400 μg/kg ivermectin, arrows) in 10 Mf carriers (Mf+) and 10 amicrofilaremic (Mf−) antigen-positive patients. Ag, Og4C3 antigenemia; GM, geometric mean.

Somatic and ES antigens from Mf. The extraction of 50,000 Mf yielded 1400 units of Og4C3 antigen (28 units/1000 Mf). This antigen was not detectable in the culture medium in which Mf were maintained for 8 days at a density of 1000 Mf/mL.

Discussion

The sensitivity of Og4C3 antigen test was absolute (100%) for patients with an Mf density >50 Mf/mL but fair (75%) for the low Mf carriers, suggesting a limit of the test to detect infections with low parasite burdens. As previously reported [1], we found a very significant but not very high correlation ($r = .59$) between antigenemia and microfilaremia, suggesting that the antigen concentration may not be only nor directly related to Mf density. The low proportion (23%) of antigen-positive patients at the end stage of disease (chronic filariasis) and the presence of Mf only in antigen-positive patients are consistent with the ability of the test to differentiate between active and past infections.

Among patients initially misclassified as Mf-negative, 73% were antigen-positive and thus were very likely infected at the beginning of the study. The initial absence of microfilaremia could be explained by the periodicity of this strain of worms or the presence of only juvenile worms at month 0. Again, in this group of patients, the antigen test did not detect persons with very low Mf density.

An important result was the consistent proportion (32%) of antigen-positive subjects without detectable microfilaremia but in whom filariasis was highly suspected on the basis of antibody results. Probably this antigen test detects prepatent asymptomatic or amicrofilaremic but infected patients for whom the parasitologic diagnosis by classic blood examination would be sought in vain. In bancroftian filariasis, most patients suffering acute clinical signs of filariasis are Mf-negative, and conversely, most of Mf carriers are asymptomatic. Anti-sheath antibodies of *W. bancrofti* were detected by IFA in 85% of the antigen-positive amicrofilaremic patients tested, compared with 2% of microfilaremic patients [5]. The presence of anti-sheath antibodies in the patients' sera has been shown to correlate with their amicrofilaremic status [6, 7]. Despite the low sensitivity of the Og4C3 antigen test to detect carriers of low-density Mf, this test should be of great benefit for the serodiagnosis of filariasis because of the stability of antigen concentration in serum [8], its ability to detect the amicrofilaremic persons, and its convenience, compared with parasitologic methods, in diagnosing the periodic nocturnal *W. bancrofti* infections. Unfortunately, this antigen was not detectable in urine samples, because of either its high molecular mass (50–60 and 130 kDa) [1] or its degradation into products that may not be recognized by the monoclonal antibody.

Even though the relationship of Mf density to adult worm burden is unclear and likely to be complicated by host immunity and the age of adult worms, the significant correlation between antigenemia and Mf count and between antigenemia and patient age jointly suggest that Og4C3 antigen is a marker of infection and of adult worm burden. This hypothesis was sustained by the significant proportion of antigen-positive persons among amicrofilaremic subjects suspected of being infected (antifilarial IgG- and IgG4-positive). The finding that the antigen level was significantly lower in Mf-negative than in Mf-positive patients may indicate a lower adult worm load in the immunologically more reactive Mf-negative patients than in the hyporesponsive Mf carriers [9].

The stability of the antigen level in diethylcarbamazine- or ivermectin-treated patients during the first hours and days of massive Mf destruction suggests that Og4C3 antigen is not a major ES or constitutive microfilarial antigen. The small amount of antigen extracted from purified Mf (28 units/1000 Mf) confirms that antigen released by dead Mf is an insignificant part of the antigen detected in serum. The absence of Og4C3 antigen in Mf culture supernatant confirmed that it is not an ES antigen, at least in the conditions of culture described. In histologic sections of adult *O. gibsoni*, this antigen has been defined as a constitutional antigen, located at the junction of the cuticle and hypodermis in the intestine cells of adults and in Mf embryos [1]. In patients treated with ivermectin, the complete dissociation between Mf count and antigenemia for 1–2 years after treatment strongly supports the hypothesis of the macrofilarial origin of this antigen and indicates the main microfilaricidal activity of ivermectin. The sustained depression of Mf count may be explained by a depression of the adult fecundity. On the other hand, diethylcarbamazine has partial macrofilaricidal activity: 6 months after a dosage of 6 mg/kg/day for 12 days, antigen level measured by another monoclonal antibody decreased by 53% [10]. Recently, we observed, at 6 months after a single 6 mg/kg dose of diethylcarbamazine (used for mass chemoprophylaxis in French Polynesia), a significant antigen decrease (>20% of pretreatment level) in 13 of 16 patients (unpublished data).

From these data, we conclude that the Og4C3 antigen detected in sera of *W. bancrofti* filariasis patients is an ES adult worm antigen. In addition to its significance as a diagnostic tool, this test will be of great value for clinical trials on new macrofilaricidal drugs and for epidemiologic studies to give insight into the dynamics of the infection in humans.

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