treatment appeared to enhance cell-mediated immunity to the OL Mf-antigen two or more weeks after treatment, possibly due to enhanced release of Mf-antigens. In studies with cynomolgus monkeys, we have noted that the OL-antigen inhibited blastogenesis in vitro (the addition of OL-antigen and Con A simultaneously to cultured normal monkey PBL reduced the response to Con A by 1/3 to 1/2), and that PBLs of monkeys injected with OL Mf were unresponsive to OL Mf-antigens but weakly responsive to *O. volvulus* adult antigens (Donnelly et al., 1988; Donnelly et al., 1986). PBLs of humans with *O. volvulus* microfilodermia showed no response against *O. volvulus* adult antigens (Gallin et al., 1988). Our present results indicate that guinea pig PBL and SL also may be affected in vivo by an inhibitory activity of OL Mf, although PHA responsiveness was largely unaffected.

The conjunctival antibody response nine weeks following rechallenge with OL Mf was enhanced. The ability of viable OL Mf in the conjunctiva to induce a prolonged local IgG and IgA antibody production and long-lasting local memory suggests that immune mechanisms involving locally produced antibody isotypes may contribute to the immunopathologic processes mediating conjunctival and corneal lesions in onchocerciasis.

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Ivermectin for treatment of bancroftian filariasis in French Polynesia: efficacy in man, effect on transmission by vector Aedes polynesiensis

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Abstract

Forty male Polynesian *W. bancrofti* carriers with mf counts ≥ 20/ml were treated with a single ivermectin 50, 100, 150 or 200 mcg/kg dose. Following therapy, mf levels fell to less than 1% of pretreatment levels in the carriers treated with the 3 highest doses. After one month, negativation rate was 40% in patients treated with a 50 mcg/kg dose, significantly lower than in patients treated with higher doses. Recurrence of microfilaremia was observed by 3 months, mf recurrence percentages were significantly lower in patients treated with the 3 highest doses than in patients treated with a 50 mcg/kg dose. At 6 months, mf recurrence percentages reached 49.8, 12.6, 14 and 5.4% of pretreatment levels in carriers treated with 50, 100, 150 and 200 mcg/kg, respectively. No significant difference was observed between mf levels by group at 6 and 12 months. With respect to efficacy, a dose ≥ 100 mcg/kg appeared superior to 50 mcg/kg dose; no significant difference between the 3 highest doses was observed. Some patients developped headache, myalgia and fever within 24 hours following therapy, none of adverse reactions were considered serious. In vector *Ae. polynesiensis* fed on carriers 6 months after treatment, average numbers of mf ingested and average numbers of L3 cephalic larvae were lower than those observed in mosquitoes fed on non-treated carriers with comparable mf counts. By reducing both mf counts in man and L3 cephalic larvae load in vector, ivermectin doses ≥ 100 mcg/kg, proved to be effective for treatment and control of filariasis due to *W. bancrofti* var. *pacifica* in French Polynesia.

Introduction

By the end of the 1940's, lymphatic filariasis due to sub-periodic *Wuchereria bancrofti* was an important public health problem in French Polynesia (Perolat et al., 1986). In the 1970's, spaced diethyl carbamazine (DEC) single-doses given either every six months or annually to the entire population resulted in reducing the microfilarial (mf) prevalence rate and density and, since 1980, mass campaigns have been routinely conducted using DEC 3 mg/kg dose given twice a year (Saugrain and Outin-Fabre, 1972; Merlin et al., 1976; Laigret et al., 1980). As a result, mf prevalence rates have fallen to 0.3–2% in the population of Tahiti, the main island, but are still high in remote islands (7–15%). Since ivermectin, a macrolide antibiotic, has been shown to be effective, first on onchocerciasis (Aziz et al., 1982) then on bancroftian filariasis (Diallo et al., 1987; Ottesen et al., 1987), a dose ranging study was carried out in French Polynesia to determine its tolerability, biological and clinical safety and efficacy in *W. bancrofti* var *pacifica* carriers. Simultaneously, an entomological study was conducted to assess the effect of Ivermectin treatment on the mf transmission by the vector mosquito, *Aedes polynesiensis* (Marks, 1951).

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Patients and methods

The study population consisted of 40 male Polynesians, between 18 and 50 years of age, in whom microfilaremia was ≥ 20 mf/ml. Main criteria for exclusions were: allergies or drug intolerance, renal or hepatic diseases and intake of antifilarial drugs during the preceeding two-year period. Carriers were explained the nature and the objective of the trial, they were given treatment and followed-up as out patients at the Public Health Clinic of Huahine, a little island near Tahiti. All biological tests were performed at the laboratory of Institut Malardé in Tahiti. For each patient, pretreatment evaluation included complete physical examination, complete blood cell count, determination of total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkalin phosphatase and creatinin levels in sera, urinanalyse, a chest radiogram and electrocardiogram. Blood mf levels were determined, using the nucleopore filtration method, before treatment and 1, 7, 14, 30, 90, 180 and 360 days thereafter. Two milliliters of heparinized blood were collected and filtered through nucleopore membran which was stained by Giemsa method for counting microfilariae.

The forty patients were divided in 4 groups and treated at one-week interval with a single dose of 50, 100, 150 or 200 mcg/kg ivermectin in same capsules containing 0.75, 1, 4 mg of ivermectin or placebo to achieve appropriate dose. They were questionned daily during 3 days regarding experience of adverse reactions which were quoted for intensity as: grade 1 – mild (easily tolerated), grade 2 – moderate (discomfort not enough to interfer with daily activity) and grade 3 – severe (subject unable to perform usual activity). The effect of ivermectin treatment of mf density was assessed according to 4 indicators: (1) the rate of successful treatment (proportion of mf-positive treated carriers in whom a reduction in mf count was observed). (2) the negativation rate (proportion of mf-positive treated carriers who became negative after treatment), (3) the decrease in mf-count observed after treatment and (4) the recurrence of microfilaremia after treatment.

Table 1 Mf counts and recurrence percentages in 40 carriers treated with single ivermectin dose

Group	no. of patients	ivermectin dose (mcg/kg)	Geometric means for mf counts and recurrence percentages (%)* pre-treatment	post treatment days 1	7	14	30	90 (%)*	180 (%)*	360 (%)*
1	10	50	648.6	8.5	2.1	1.4	1.8	167.4 (25.8)	323.1 (49.8)	352.3 (54.3)
2	10	100	469.6	4.7	0	0.5	0	23.6 (5)	59.2 (12.6)	82.6 (17.6)
3	10	150	842.8	6.2	0.3	0	0	33.7 (4)	118.6 (14)	430.7 (51.1)
4	10	200	720.5	0.9	0.1	0.1	0.5	32.6 (4.5)	38.7 (5.4)	189.3 (26.3)

* Percent of pretreatment geometric means

Table 2 Average number* of ingested microfilariae and average number* of cephalic infective larvae (L3) in *Ae. polynesiensis* fed on non-treated carriers and on carriers six months after treatment with a 200 mcg/kg ivermectin single dose

mf carriers	mf density (nucleopore)	no. of mosquitoes dissected	percentage infected mosquitoes	average no. of larvae ingested	range of larvae ingested	average no. of cephalic L3
non-treated	1–99	251	56.17	8.76	1–54	4.09
	> 1000	1481	94.60	38.44	1–461	5.81
6 months after treatment	1–99	344	20.93	1.68	1–11	0.4
	> 1000	316	77.22	7.65	1–34	2.04

* Mean of average larvae numbers per batch of mosquitoes fed on one carrier

More than 500 laboratory-bred *Ae. polynesiensis* were fed on one arm of 8 of the 10 carriers treated with a single ivermectin 200 mcg/kg dose and on 5 non treated carriers with comparable mf densities, the day before and 30, 90, 180 and 360 days after treatment. At each study day, for each subject, batches of more than 30 mosquitoes were dissected immediately after feeding and then, daily during 14 days. The effect of ivermectin on the transmission by vector was assessed on (1) the average number of mf ingested and (2) the average maximum number of larvae reaching the infective stage (L3 cephalic larvae). This paper deals only with the results observed six months after treatment.

Statistical analysis were by Pearson's chi square test, Student's t test, Mann and Whitney's U test (Schwartz, 1981). For comparison, mean mf-counts by group of carriers were expressed in geometric means.

Results

In man

Before treatment, individual mf levels ranged from 30 to 3000 mf/ml, geometric means for mf counts of the 4 groups (648.6, 469.6, 842.8 and 720.5) were not significantly different ($p > 0.05$). Following treatment, mf levels dropped rapidly in all of the patients (successful treatment rate 100%). Clearance of microfilaremia was not complete in all of the patients: by day 30, the negativation rate was 40, 100, 100 and 90%, respectively, in group 1, 2, 3 and 4, the mf decrease was more than 98% in group 1, 100% in groups 2, 3 and more than 99% in group 4 (Table 1). Recurrence of microfilaremia was observed 3 months after treatment, it was 25.8% of the pretreatment level in group 1, significantly higher ($p < 0.05$) than the 5, 4 and 4.5% recurrence percentages observed in the groups treated with the 3 highest doses. Conversely, no significant difference ($p > 0.05$) was observed between these latter 3 groups. Six and twelve months after treatment, recurrence percentages have reached 49.8, 12.6, 14 and 5.4%; 54.3, 17.6, 51.1 and 26.3% of pretreatment levels in groups 1, 2, 3 and 4. At 6, 9 and 12 months, no significant difference ($p > 0.05$) was observed between the 4 groups, either in geometric means for mf counts or in mf recurrence percentages.

Twenty nine carriers experienced some side effect, mainly fever, headache, myalgia and cough, which appeared in 12 to 24 hours after the intake of treatment, peaked in intensity between 24 and 48 hours and disappeared by 72 hours. The side effects were mild in 13 subjects, moderate in 14 and severe in the last 2 (both treated with 150 mcg/kg dose) who suffered intense headache and myalgia with a fever of more than 40 °C. All reactions were easily managed with administration, when required, of antalgic and antipyretic drug (paracetamol). With regard to frequency, no difference was found between the 4 groups ($p > 0.05$); with regards to intensity, grade 2 and 3 reactions were more frequent in group 2 and 3 as compared to group 1 and 4 ($p < 0.05$). Finally, side reactions were significantly more frequent ($p < 0.05$) in subjects with high mf levels ($\geq$ 1000 mf/ml) than in subjects with low mf levels (< 1000 mf/ml).

In vector

Six months after the intake of the drug, mf counts ranged from 0 to 99 mf/ml in 5 of the 8 carriers treated with ivermectin 200 mcg/kg and were > 1000 mf/ml in the 3 others. The percentage of infected mosquitoes was 58.17 and 94.60%, respectively, when fed on non-treated carriers with mf counts of 0–99 mf/ml and > 1000 mf/ml; it was 20.93 and 77.22% when mosquitoes were fed on treated carriers with comparable mf counts. The 8.76 ± 6.5 average number of microfilariae ingested (mean of average mf numbers per batch of mosquitoes fed on one subject) by mosquitoes fed on non-treated subjects with mf counts of 0–99 mf/ml did not differ significantly ($t = 2.17$, $0.05 < p < 0.1$) from the 1.68 ± 0.52 average number ingested by mosquitoes fed on the 5 carriers with comparable mf counts 6 months after treatment (Table 2). Conversely, a significant difference ($t = 2.93$, $p < 0.05$) was found between the average number of ingested microfilariae in mosquitoes when fed on non-treated carriers with mf counts of more than 1000 mf/ml (38.44 ± 21.04) and when fed on the 3 carriers with comparable mf counts six months after treatment (7.65 ± 0.7).

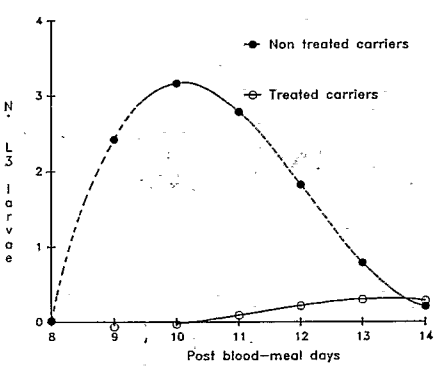

Fig. 1 Average numbers of cephalic L3 larvae in mosquitoes fed on treated and non-treated carriers with 0–99 mf/ml

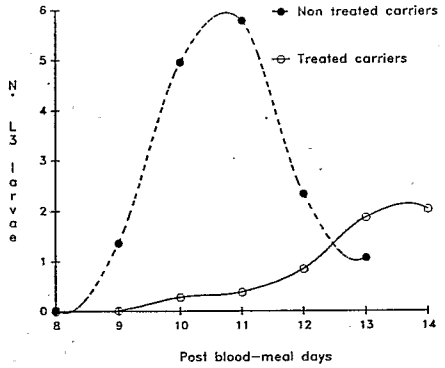

Fig. 2 Average numbers of cephalic L3 larvae in mosquitoes fed on treated and non-treated carriers with > 1000 mf/ml

The 4.09 ± 2.97 average number of infective cephalic L3 larvae (mean of average numbers of L3 per batch of mosquitoes fed on one subject) in mosquitoes fed on non-treated subjects with mf counts of 0–99 mf/ml did not differ significantly from the 0.4 ± 0.5 cephalic L3 average number in mosquitoes fed on the 5 subjects with comparable mf counts 6 months after treatment ($t = 2.45$, $0.05 < p < 0.06$). Conversely, the 5.81 ± 3.1 average number of L3 cephalic larvae in mosquitoes fed on non-treated subjects with mf counts of more than 1000 mf/ml differed significantly ($t = 2.35$, $p < 0.05$) from the 2.04 ± 0.88 average number of L3 cephalic larvae in mosquitoes fed on the 3 subjects with comparable mf densities 6 months after treatment. Average numbers of infective L3 larvae we have reported were the maximum numbers, they were observed on day 10 in mosquitoes fed on non-treated subjects with mf counts of 0–99 mf/ml, and on day 13 in mosquitoes fed on carriers with comparable mf densities 6 months after treatment (Fig. 1). In mosquitoes fed on non-treated subjects with mf counts > 1000 mf/ml, the maximum average number of L3 was observed on day 11 when it was observed on day 14 in mosquitoes fed on carriers with comparable mf densities 6 months after treatment (Fig. 2). The 10–11 days developmental period in *Ae. polynesiensis* fed on non treated persons was comparable to the 12-day period observed by other workers, either in French Polynesia (Rosen, 1955) or elsewhere in the South Pacific (Bryan et al., 1976). Though the small number of carriers participating to the entomological study did not allow to show significant difference, the period of time required for larvae to reach the infective stage (developmental period) was longer in mosquitoes fed on treated subjects than in mosquitoes fed on non-treated subjects with comparable mf counts.

Discussion

Our results clearly indicate that ivermectin single-dose treatment is effective on lymphatic filariasis due to *W. bancrofti* var. pacifica. The successful treatment rate was 100%, however, the four doses did not show similar microfilaricidal activity. After one month, the negativation rate was nearly 100% in the three groups treated with the highest doses when it was only 40% in the group treated with a 50 mcg/kg dose. After 3 months, the mf recurrence percentage was lower in the groups treated with the three highest doses than in the group treated with 50 mcg/kg dose. This difference, though not observed after 6 and 12 months, together with the significantly lower negativation rate in group 1 suggest that a single ivermectin 50 mcg/kg dose should not be recommended for treatment of lymphatic filariasis in French Polynesia. Since efficacy of the 3 highest doses in reducing microfilaremia did not differ significantly, one may assume that a 100 mcg/kg dose could be the minimal effective dose. Moreover, since geometric means for mf levels were not significantly different at six and twelve months in any of the 4 groups, the possible efficacy of annual ivermectin single-dose treatment in lymphatic filariasis might be considered.

Side effects were observed in about 70% of the carriers; in fact, two carriers only were unable to perform usual activities for less than 3 days and, in neither case, reactions were considered serious. Relationship between ivermectin dose and intensity of side-reactions was not clear, the fact that reactions were more severe in group 2 and 3 as compared to group 1 and 4 is likely to be due to the small number of patients by group. Relationship between intensity of side-effects and pretreatment mf levels was more clear. Our results, as those reported by other workers using either DEC (Galliard, 1958; Kimura et al., 1985) or ivermectin (Kumaraswami et al., 1988) for treatment of lymphatic filariasis, suggest that side-effects could be more closely related to the parasite killing than to the toxicity of drug.

The decrease of both the number of microfilariae ingested and the number of larvae reaching the infective stage together with the increase of the period of time required for larvae to reach the infective stage in *Ae. polynesiensis* fed on treated carriers, even 6 months after treatment, indicate that a single 200 mcg/kg ivermectin dose administered to the carriers resulted in reduction of the transmission by vector.

Further studies are needed to assess whether similar results are observed in mosquitoes fed on carriers treated with lower doses. Comparable observations have been made in mosquitoes fed on carriers treated with DEC, first by Laigret (1965) on the basis of epidemiological analysis, then by Chen and Fan (1977) in entomological study carried out in *Culex quinquefasciatus* and by Samarawickrema (1985) in *Ae. samoanus* and *Ae. polynesiensis*.

The decrease of both mf counts in man and L3 cephalic larvae numbers in vector has been estimated to give a good picture of the efficacy of antifilarial treatment (Kessel, 1971). According to those criteria, ivermectin seems to have great potential for the treatment of bancroftian filariasis in French Polynesia. In vector, a decrease of infective worm load in mosquitoes fed on patients treated with the highest ivermectin dose was observed; in man, at the three highest doses, a single intake of ivermectin induced immediate clearance of about 100% of microfilaremia and sustained the mf decrease of about 85% of pretreatment mf levels till 6 months. A filariasis control strategy based on administration of spaced single ivermectin doses would have great advantages in simplicity and low cost as compared to the currently recommended total dose of 72 mg/kg of DEC (Expert Committee on Filariasis, 1984; Mak, 1987). As compared to the present filariasis control strategy in French Polynesia, single ivermectin dose treatment would have advantage in inducing immediate mf clearance much higher than the 15% clearance usually observed in *W. bancrofti* var *pacifica* carriers after a single DEC 3 mg/kg dose (Cartel, 1990). Nevertheless, relative effect of ivermectin and DEC as well on microfilaremia in Polynesian subjects as on transmission by vector should be compared in a blind-study including the administration of single-dose of DEC.

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Cloning of specific diagnostic antigens of Onchocerca volvulus

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Abstract

Specific, serological diagnosis is one of the main goals in onchocerciasis research. To date this objective has been hampered by (a) scarcity of parasite material, and (b) antigenic cross-reaction between *Onchocerca volvulus* and other nematode species. In order to obtain specific antigens, and in amounts suitable for study, molecular biological techniques have been adoped. A lambda gt11 cDNA expression library prepared from *O. volvulus* adult female worms was screened using infected human sera from onchocerciasis patients and rabbit hyperimmune sera raised against *Onchocerca* and genus-specific *Onchocerca* antigen extracts. Five clones were selected and their inserts expressed as β-galactosidase fusion proteins. The fusion proteins were examined using individual sera from patients with *O. volvulus* or *Wuchereria bancrofti* infections. Three of the fusion proteins were recognised by more than 80% of *O. volvulus* sera and exhibited weak reactivity with a few *W. bancrofti* sera. One of these three clones was recognised to a significantly greater degree by sera from sowda than from generalised onchocerciasis patients.

Introduction

Diagnosis of onchocerciasis is currently dependent upon parasitological and serological techniques, both of which show serious limitations. Thus, parasitological techniques exhibit low sensitivity, fail to detect prepatent and infections with low filaria loads, require special expertise and are time consuming (Taylor et al., 1989; Albiez et al., 1988). With respect to serological methods, the common use of unfractionated cross-reacting antigen extracts, usually dictates that they are of low specificity (Cabrera et al., 1989). Resolution of this latter problem by the preparation of purified specific antigens is hampered by scarcity of parasite material (Philipp et al., 1984).

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We, like others (Lucius et al., 1988b), have therefore turned to molecular cloning as a way of producing such molecules. In this paper, we compare the specificity properties of five recombinant polypeptides, each selected by a different strategy.

Material and methods

Sera

Bancroftian filariasis patient sera were provided by the WHO Filariasis Serum Bank, Geneva, and by Dr. Shatry, Kenya. Onchocerciasis sera from Yemen and Liberia were collected in the course of field studies (D.W.B.). A serum from Venezuela was kindly provided by Dr. Z. Cabrera and Dr. L. Yarzabal, Puerto Ayacucho and a serum from Mexico was collected by Dr. Gomez-Priego, Chiapas.

Rabbit serum R-338 was a hyperimmune serum prepared against *Onchocerca gibsoni* microfilariae, rabbit serum R-384 was raised against a low molecular weight surface extract of adult *O. volvulus* (Fraction II) (Cabrera and Parkhouse, 1987).

Differential screening of a cDNA O. volvulus library

A lambda gt11 cDNA library derived from adult female *O. volvulus* isolated in Kumba, Cameroon, was provided by Dr. J. Donelson, University of Iowa, USA (Donelson et al., 1988). The screening of the library was carried out using the strategies illustrated in Table 1. The sera used included infected human serum pools from patients with generalised onchocerciasis (n = 35), sowda (n = 20) or bancroftian filariasis (n = 10). Bound human total Ig, IgG4 and IgE were revealed with goat-antihuman Ig-peroxidase (LC.N. Pharmaceuticals), ^{125}I-labelled mouse monoclonal anti-human IgG4 (JDL), and ^{125}I-labelled mouse monoclonal antihuman IgE (E-RB6-2) respectively (Cabrera et al., 1986). Rabbit sera, including anti-*O. gibsoni* anti-purified surface antigen (Fraction II) (Cabrera and Parkhouse, 1987) and anti-*O. gibsoni* uterine microfilaria PBS extract were

Table 1 Strategies used in differential library screening in order to isolate *O. volvulus* specific clones*

Clone type	Serological systems
I	Generalised onchocerciasis-anti-human IgE +ve/ anti-human IgG4−ve
II	Generalised onchocerciasis +ve/Bancroftian filariasis−ve
III	Sowda onchocerciasis +ve/generalised onchocerciasis−ve
IV	Anti-*O. gibsoni* Fraction II +ve/Bancroftian filariasis−ve
V	Anti-*O. gibsoni* MF +ve

* Pooled human sera from infected persons were used in the isolation of clones I, II and III. Clones IV and V were identified with hyperimmune rabbit sera raised against fraction II of an *O. gibsoni* extract (4) and *O. gibsoni* Mf respectively