Efficiency of papain-treated microfilariae of *Wuchereria bancrofti* (var. *pacifica*) as antigen for serodiagnosis of bancroftian filariasis in French Polynesia

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Abstract

Papain-treated microfilariae of *Wuchereria bancrofti* have been used as antigen for indirect fluorescent assay: 0% of non-endemic sera, 8% of healthy exposed Polynesians, 48% of clinical patients and 96% of microfilaraemic subjects were positive by this test. The geometric mean titres were 22, 41, 147 and 605 respectively. Untreated microfilariae were unsuitable for diagnostic purposes. *Dirofilaria immitis* adult sections showed low reactivity, giving poor discrimination between non-endemic and microfilaraemic sera. The geometric mean titres were 6 and 61 respectively.

Introduction

Wuchereria bancrofti (var. *pacifica*) is the only filarial parasite of man in French Polynesia. The prevalence of this disease estimated by parasitological methods has fallen from 30% to less than 3% of the population, owing to a continuing prevention programme for the past 35 years.

Bancroftian filariasis has a wide clinical spectrum, from the severe state of elephantiasis to the asymptomatic presence of microfilariae (mff) in the blood. The usual clinical course is characterized first by the asymptomatic, then the acute and finally the chronic stage when the adult worm is dead. The adult worm may last longer than ten years (CARME & LAIGRET, 1979) and the mechanism for this persistence is not yet clearly understood.

With the presently available methods, it is difficult to diagnose filariasis unless mff are found, and impossible to detect subpatent and subclinical infection. The humoral response to filarial antigen has a wide range of level, and there is no correlation between antibody response and the severity of the infection. Anti-microfilarial sheath antibodies are not usually detected in microfilaraemic patients, conversely to the clinical forms of the disease (SUBRAHMANYAM *et al.*, 1978; PIESSENS *et al.*, 1980; MCGREEVY *et al.*, 1980). With papain-treated mff, other antigenic sites are exposed and very high antibody titres are detected in asymptomatic microfilaraemic subjects by means of an indirect fluorescent assay (GONZAGA DOS SANTOS *et al.*, 1976; CHANTEAU & DURSOIR, 1985). These antibodies are probably just a sign of the infection, whereas anti-sheath antibodies promote leucocyte adherence and cytotoxicity. The possibilities of the papain-treated mff test being used for diagnosis are analysed in the present paper. The results are compared with those obtained with untreated mff antigen and sections of adult *Dirofilaria immitis*.

Materials and Methods

1. *Sera*

Four groups, each of 50 sera, were constituted:

Group I — newcomers from a country free from filariasis;

Group II — healthy exposed Polynesians, showing no microfilaraemia and no clinical features of filariasis;

Group III — patients with clinical filariasis (lymphadenitis, lymphangitis, elephantiasis, chyluria, hydrocele . . .) but showing no microfilaraemia. In this group we included newly diagnosed patients as well as patients with chronic infection treated by our Institute for more than 10 years;

Group IV — asyptomatic microfilaraemic patients.

All the sera were stored at −20°C until use.

2. *Counting microfilariae*

5·0 ml of blood collected in citrate anti-coagulant were mixed with 10 ml saponin (2%) and 10 ml distilled water to induce haemolysis. After centrifuging for 10 min. at 600 *g*, the sediment was washed twice with NaCl (0·15 M) and the number of mff counted under the microscope.

3. *Collection of* W. bancrofti *var.* pacifica *microfilariae*

Microfilariae were isolated as follows: 5·0 ml of phytohaemagglutinin P (Bacto) were added to 50 ml of citrate blood half diluted with NaCl (0·15 M) to allow cell agglutination. The mixture was carefully layered over 100 ml of Percoll d = 1·08 (Pharmacia) contained in a 250 ml transfusion flask. After centrifuging for 20 min. at 600 *g*, the whole supernatant containing the mff was transferred into another 250 ml flask and washed three times with NaCl (0·15 M) by successive centrifugation for 20 min. at 800 *g*. The purified mff, still alive, were frozen in aliquots at −20°C.

4. *Preparation of the antigen slides*

4.1. Microfilariae slides (WB)

The purified mff were thawed and adjusted to 20 mff per 25 µl with phosphate buffered saline (PBS 0·01 M, pH 7·2). The suspension was distributed with a dropper into the wells of slides (diameter of the well = 7 mm). The slides were allowed to dry at 37°C, and stored at −20°C.

4.2. The papain treated microfilariae slides (WBp)

The papain digestion was carried out as follows: 2 ml of mff suspension were added to 4 ml of papain cysteine solution prepared just before use (one volume papain 0·5% (P 4762-Sigma) + one volume $Na_2HPO_4.2H_2O$, 1·8% + one volume cysteine chlorydrate 0·2%). After five minutes' incubation at 37°C, the reaction was stopped at 4°C in an ice bath. Papain was removed by two washings with cold PBS with successive centrifugation at 4°C, 10 min at 800 *g*. Adjusted to 20 mff per 25 µl, the antigen thus obtained is used for slide preparation as described above.

4.3. Dirofilaria immitis *sections (DI)*

Adult *D. immitis* were cut in frozen sections 5 μm thick with a cryostat, and stuck on to slides. After 10 min fixation in cold acetone, the slides were kept at −20°C.

5. *Indirect fluorescent assay (IFA)*

The sera were diluted two-fold (1/10, 1/20 . . .) with PBS, pH 7·2 containing Tween 20, 0·1%. One drop of each dilution was placed in each well of the slides, incubated 30 min at room temperature and washed twice with PBS. The fluorescent anti-human immunoglobulin, diluted with PBS + Evans Blue 1/10,000, was then applied, incubated and washed in the same way. The slides were mounted in buffered glycerin and examined under a fluorescent microscope (Zeiss, × 100 magnification).

For convenience, the fluorescent assays with the three antigens were designated: IFA-*WB*, for *W. bancrofti* untreated microfilariae; IFA-*WBp*, for *W. bancrofti* papain-treated microfilariae; IFA-*DI*, for *D. immitis* adult sections.

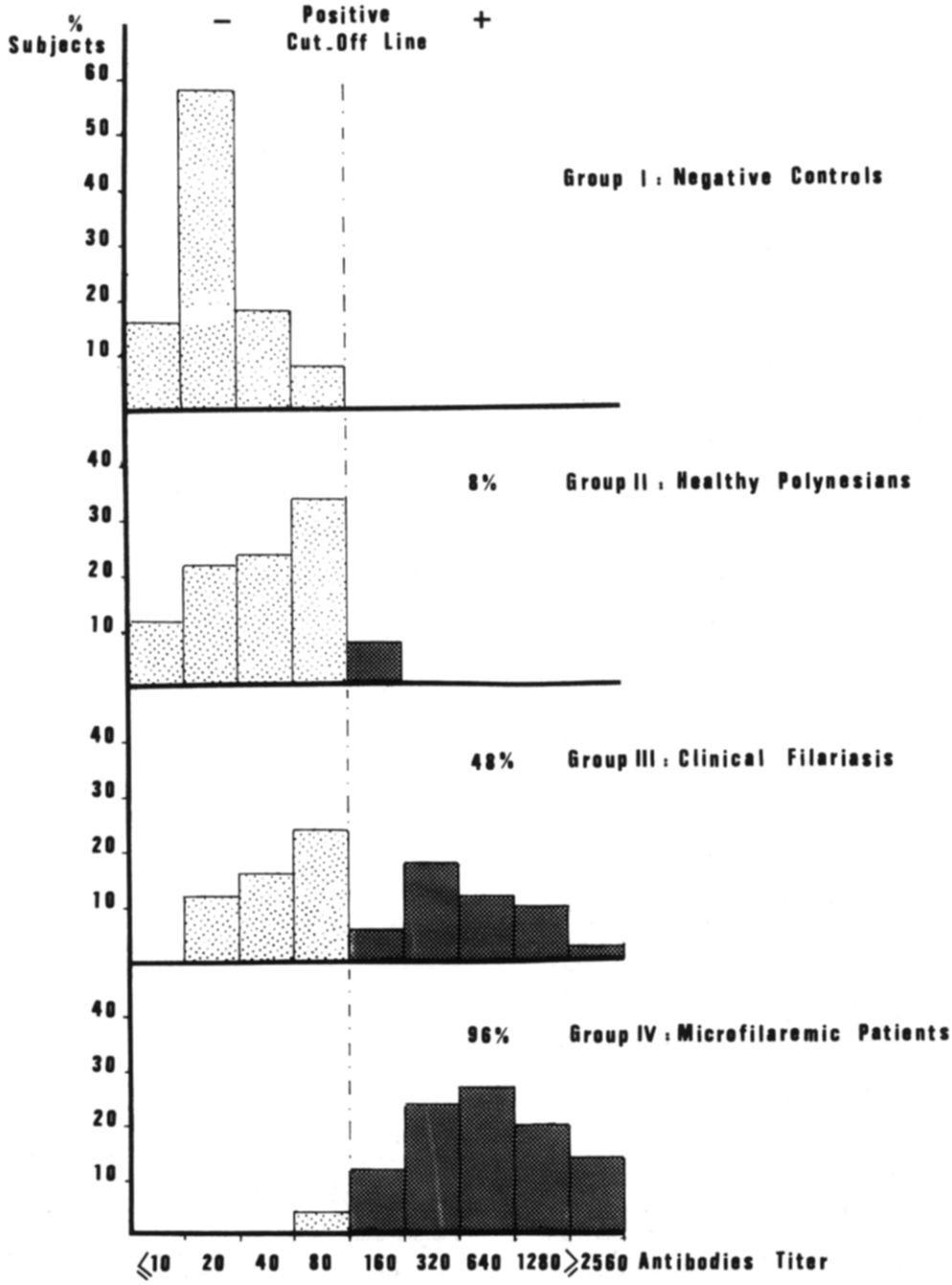

Fig. 1. IFA-*WBp* titre distribution of the four groups of sera.

Results

1. The indirect fluorescence assay with papain-treated microfilariae IFA-*WBp*.

Sera of the four groups were tested by end-point titration and the statistical distribution of their titres is represented in Fig. 1. The positive base-line was determined after taking into account results obtained with the negative controls (Group I). Thus, a titre ≤ 80 was considered negative and represents non-specific antibodies, whereas a titre > 80 was considered positive for filarial infection. According to this classification, we obtained: 0% positive in the negative controls (geometric mean titre = 22·6); 8% positive in the healthy Polynesians showing no evidence of filariasis — the maximum titre obtained for these positive sera was 160 (geometric mean titre = 41·1); 48% positive in the patients with lymphatic pathology and no circulating mff (geometric mean titre = 147·2); the histogram suggests heterogeneity in the population and the significance of these results will be discussed; 96% positive in the asymptomatic microfilaraemic subjects, 60% having a titre ≥ 640 (geometric mean titre = 605·4).

2. Comparative serology using IFA-*WB*, IFA-*WBp* and IFA-*DI*.

The three fluorescent assays were applied to each group of sera. The cut-off line, determined from results of negative controls, was different for each test. The cut-off titres were ≥ 10 for IFA-*WB*, > 80 for IFA-*WB*-*WBp* and ≥ 40 for IFA-*DI*.

The percentage of positive sera in each group was calculated and is reported in Table I. In healthy Polynesians, 4 to 8% of people showed a weak but positive reaction with the three tests. They may be assumed to represent the high risk population.

In the group with clinical filariasis, half was positive with IFA-*WB* and IFA-*WBp*, whereas IFA-*DI* detected 78%. The negative results were obtained mainly from old patients who had been treated for 10 to 30 years, but also from some relatively new cases (three to six years' treatment) and a few doubtful cases presenting other pathology simultaneously.

In the asymptomatic carriers, only 2% were detected by IFA-*WB*, whereas 96% and 92% showed a significant level of antibodies with IFA-*WBp* and IFA-*DI*.

IFA-*WBp* and IFA-*DI* gave a similar rate of positives in all the groups, although in the group of clinical patients anti-*DI* antibodies were present in most of the sera. A further comparison of these two tests, by analysis of the titre distribution (Fig. 2), showed a better discrimination between negative and microfilaraemic subjects with IFA-*WBp*. Geometric mean titres for controls and patients were 6·2 and 61·5 for IFA-*DI*, and 22·6 and 605·4 for IFA-*WBp*, respectively.

Discussion

We previously reported the usefulness of papain-treated *W. bancrofti* mff as antigen for detecting anti-filarial antibodies in microfilaraemic patients infected with homologous parasites. The test IFA-*WBp* had also shown significant reactivity with sera of individuals infected with *Onchocerca volvulus*, *Brugia malayi* and *Loa loa* but low reactivity with some common intestinal parasites, e.g., *Ascaris lumbricoides* (see CHANTEAU & DUROSOIR, 1985). The non-specific interaction is cut off by fixing the positive base-line at a titre > 80, so that non-endemic sera were all negative. In a population of normally exposed Polynesians, 8% are found positive for filariasis using IFA-*WBp*. The possibility of subclinical filariasis is suggested, but since no mff and no clinical features were observed, only future surveillance of these subjects could confirm the diagnosis. Repeated challenge by antigen in endemic areas has also to be considered. This second hypothesis is supported by the low antibody titre obtained with those sera (titre = 160). The low percentage positive in the normal Polynesians reflects the low filarial endemicity of the region: the actual prevalence of filariasis, determined by the presence of mff in thick capillary blood films, is around 3% in French Polynesia, and less than 1% in Tahiti Island. But it is more than likely that the real prevalence is higher, if more discriminative techniques were used.

The results obtained with the patients showing clinical disease without microfilaraemia are interestingly heterogeneous. It is known that the chronic

Table I—Serology of the different groups of sera using IFA-*WB*, IFA-*WBp* and IFA-*DI*

Groups	No. of sera	IFA-*WB* Percentage positive	IFA-*WB* Geometric mean titre	IFA-*WBp* Percentage positive	IFA-*WBp* Geometric mean titre	IFA-*DI* Percentage positive	IFA-*DI* Geometric mean titre
I : non-endemic subjects	50	0%	0	0%	22·6	0%	6·2
II : normal exposed Polynesians	50	6%	1·1	8%	41·1	4%	3·1
III: clinical filariasis patients. Mff negative	50	54%	7·6	48%	147·2	78%	45·9
IV: asymptomatic microfilaraemic patients	50	2%	1	96%	605·4	92%	61·5

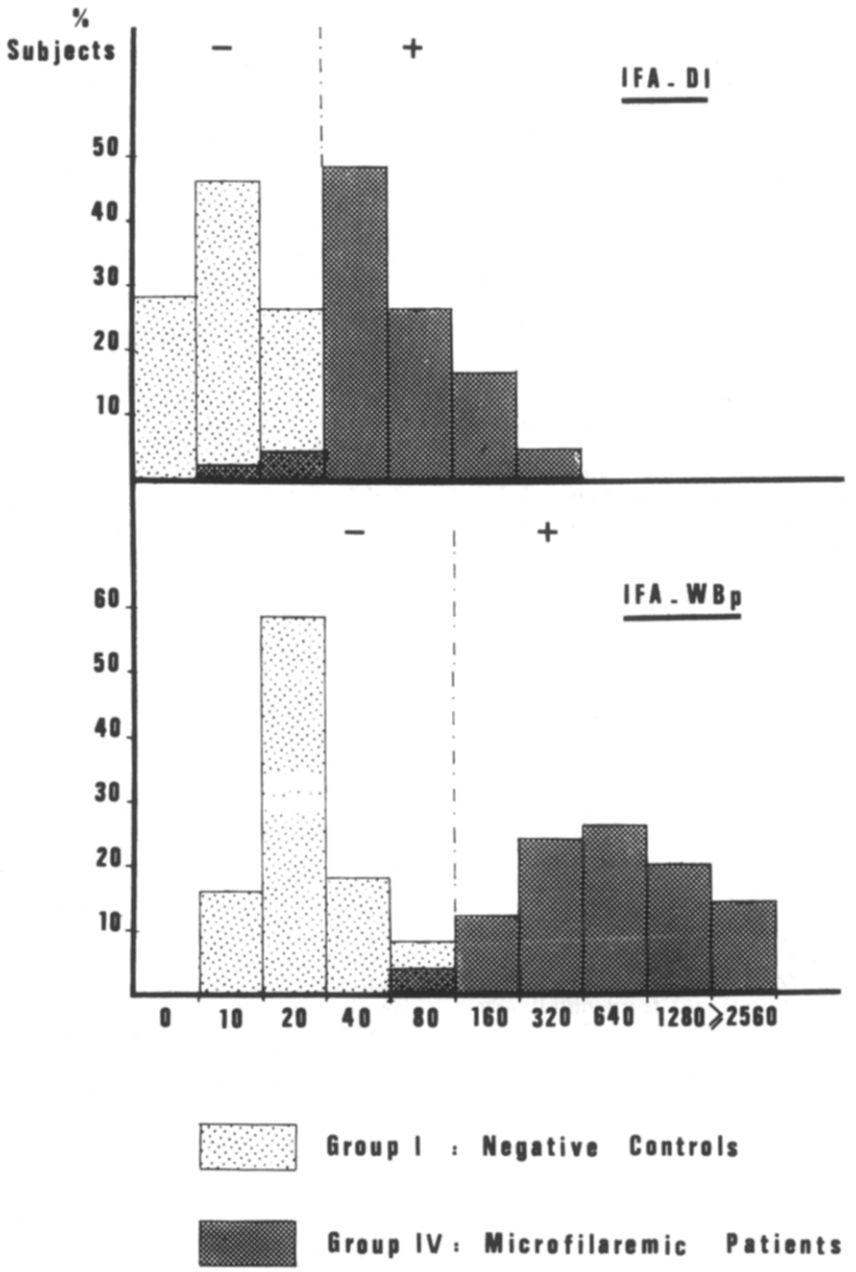

Fig. 2. Efficiency of IFA-*DI* compared to IFA-*WBp* for the distinction of negative and microfilaraemic controls. Geometric mean titres are 6·2 and 61·5 for IFA-*DI*; 22·6 and 605·4 for IFA-*WBp*.

stage of filariasis is an end stage when adult worms are dead but obstructive pathology persists. It is also generally admitted that those individuals are exposed to the same risk of re-infection as the general population (PARTONO, 1984). Our results tend to show that effectively the patients examined can be classified in two groups of active infection and chronic end-stage infection, corresponding respectively to the positive and negative groups of the histogram. The clinical history of these subjects correlates well with their serology. Among the group of sero-negative individuals, the presence of non-filarial lymphangitis is possible, as no mff were found. A microbial or post-traumatic aetiology has to be considered, especially in a barefoot community.

Among the asymptomatic microfilaraemic sera, 96% had a very high antibody level (geometric mean titre = 605·4). The papain-treated mff antigen is

likely to be of great use in the diagnosis of low-level mff carriers, when classical techniques cannot demonstrate mff. This possibility is particularly interesting as *W. bancrofti* var. *pacifica* has no established periodicity, so the chance of finding mff cannot be focused on time, as can be done for the nocturnally periodic species.

Other authors have used soluble or sonicated microfilarial antigens for immmunodiagnosis of filariasis. KALIRAJ *et al.* (1981) have shown that soluble antigens lack specificity while recently NAIDU *et al.* (1984) demonstrated a relative efficacy of this antigen using the indirect haemagglutination assay test: 71% of microfilaraemics, 57% of acute cases, 53% of chronic cases and 33% of endemic normal persons could be detected. Some cross reactions were observed with hookworms and roundworms.

The comparative study of IFA-*WB*, IFA-*WBp* and IFA-*DI* shows that untreated mff are of no use as antigen for diagnosis. The heterologous antigen from *D. immitis* showed efficiency similar to that of papain-treated mff but, owing to its low reactivity, narrow titre variation between negative and positive sera, difficulties of reading and laborious antigen preparation, IFI-*DI* is not convenient for routine analysis.

In conclusion, the use of papain treated mff as antigen in the indirect fluorescent assay seems to be useful for sero-epidemiological studies and the diagnosis of bancroftian filariasis. In association with the parasitological technique of haemoconcentration, it is actually used satisfactorily for the individual diagnosis of filariasis in French Polynesia.

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