

A new immunodiagnostic test in onchocerciasis*

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Summary

The radioallergosorbent (RAST) technique was used as a diagnostic test for onchocerciasis. IgE antibodies for *Dirofilaria immitis* antigen were found in thirty-one of fifty-seven (54%) affected patients.

Introduction

Immunodiagnosis of parasitic infections is well accepted in several tropical diseases, in malaria (Voller *et al.*, 1975) in Schistosomiasis (Madwar & Voller, 1975), in *Toxocara* infection (Hogarth-Scott, Johansson & Bennich, 1969; Kagan, 1968), in filariasis (Ciferi *et al.*, 1975), in amoebiasis and in onchocerciasis (Mueller, Jerks & Fruitstone, 1973), but not for the *Onchocerca volvulus* worm. The immunological methods used in assessing onchocerciasis are skin testing (Ikejiani, 1954) precipitation (Ulrich, Pinnardi & Convit, 1970) and immunofluorescent techniques. In a previous study it was shown that 80% of fifty-seven adult Nigerian patients with onchocerciasis had increased total serum immunoglobulin E (Somorin, Ajugwo & Heiner, 1975). The radioallergosorbent technique (RAST) of Wide, Bennich & Johansson, (1967) for detection of specific antibodies was used to identify filarial antibodies in sera of onchocerciatric patients, and the present study reports IgE antibodies to *Dirofilaria immitis* in the sera of onchocerciatric patients.

Materials and methods

Criteria for inclusion in the study

All patients in the study were clinical cases of onchocerciasis based on the WHO diagnostic criteria of Buck (1973) namely, pruritus, dermatitis, onchocercomata, lymphadenopathy, hanging groin sign, oedema of the legs with or without optharmo-

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logical or systemic manifestations. Dermal microfilaria and eosinophilia may or may not be present. All patients were screened to exclude other causes of eosinophilia.

Nigerian sera

Sera of patients were frozen and transported from Lagos, Nigeria to Torrance, California within 24 hr.

Nigerian and American control sera

The serum of an allergic adult Nigerian patient whose total serum IgE had been previously measured (Somorin *et al.*, 1975) was also included in the study. The pooled sera of adult allergic Californian American patients whose total serum IgE has been measured previously and standardised against the WHO IgE, reference standard 15818 (1 iu/ml = 2 ng/ml) was also used as a control serum.

Antigen

The antigen was prepared from dried lyophilized *Dirofilaria immitis* (a filarial worm of dog's heart muscle) antigen (supplied by Dr Irving Kagan of the Centre for Disease Control, Georgia). The saline extract of the antigen was dialysed against 200 ml of 0.5 M bicarbonate buffer, pH 8.4 for 24 hr at 4°C. The protein concentration of the antigen dialysate was determined from the optical density based on extinction coefficient of 1.5 and using a Beckman spectrophotometer.

Cyanogen bromide activation of paper discs

Twenty grams of paper discs were washed three times in 200 ml of de-ionised water, 25 g of cyanogen bromide, was added, under a fume hood, to the washed paper discs. The pH was immediately brought to 10.5–11 by addition of 2 N NaOH and was kept at this pH until 100 ml of the caustic soda had been used, when the liquid was drawn off. The discs were washed twice with 500 ml of 0.005 M sodium bicarbonate and then twice with 50% acetone. The discs were then dried.

Radiolabelling of anti-IgE

Rabbit antiserum to IgE (ND) had previously been prepared by one of us (D.C.H.). The technique of radiolabelling has been described previously (Hunter & Greenwood, 1962; Somorin *et al.*, 1976).

*Coupling of *Dirofilaria immitis* antigen to cyanogen bromide-activated paper discs:*

The *Dirofilaria immitis* antigen was mixed with 300 mg of paper discs. This was agitated for 48 hr after which the discs were washed three times with 0.5 M sodium bicarbonate buffer, pH 8.4, they were then suspended in 0.2 methanolamine in 0.1 sodium bicarbonate and were agitated for an additional 24 hr. The supernatant was then removed, the discs were washed with 0.5 bicarbonate, pH 8.4 and two washes with incubation buffer. The *Dirofilaria immitis* antigen-coupled activated paper discs were stored in incubation buffer at -4°C until ready for use.

Method

*Detection of *Dirofilaria immitis* IgE antibodies in patients with *Onchocerciasis volvulus**
Incubation buffer, 0.05 ml (500 ml M sodium phosphate, 9 g sodium chloride, 12 ml of

20% human serum albumin, 0.49 g of sodium azide, and 5 ml of Tween 20), was added to each tube containing allergen-coupled or plain activated paper-discs and 0.01 ml of the serum. After 4 hr incubation at room temperature and slight shaking, the mixture was washed three times with 2.5 ml of normal saline. Each remaining paper disc was then incubated with 0.1 ml of ^{125}I anti IgE having a radioactivity of 1 000 000 ct/min. The procedure was carried out in duplicate.

The total radioactivity was determined on an Atomic Baird Gamma Counter Model 530. The bound radioactivity was similarly counted after washing each serum-incubated mixture three times with 2.5 ml normal saline. The percentage IgE bound was then expressed 0 to 4+.

Results

Thirty-one of the fifty-seven patients (54.3%) had positive RAST; of these thirteen (22.8%) had a 1+ positive test, i.e. small amounts of specific IgE anti-*Dirofilaria immitis*, according to the binding capacity of the antigen (Table 1); ten (17.5%) had a 2+; and eight (14%) had 3 or 4+ plus, i.e. large amounts of specific IgE antibodies. Since RAST showed low counts in the pool of sera from allergic Americans, or the one allergic Nigerian, it is assumed that the positive RAST indicate specific antibody to *Dirofilaria immitis*.

Table 1. Interpretation of positive grading in the study

Percentage maximum binding capacity	RAST grading
15-24	+
25-35	++
36-44	+++
Over 45	++++

Discussion

Onchocerciasis, an endemic disease of the equatorial zone, is characterized by dermatological, ophthalmological, lymphatic and systemic manifestations (Buck *et al.*, 1969). Although the disease has a low mortality, its morbidity is quite high (WHO, 1962). The immunological basis for this high morbidity is being investigated. In this study, of the fifty-seven onchocerciatric patients studied, thirty-one patients (54.3%) were RAST positive i.e. demonstrated specific IgE antibodies to *Dirofilaria immitis* antigen. Twenty-eight onchocerciatric patients were RAST negative with no specific IgE *Dirofilaria immitis* antibodies. The fact that an allergic Nigerian serum and pooled allergic Californian American serum had no specific IgE antibodies to *Dirofilaria immitis* antigen does not exclude false positive results which might be due to heterophile antibodies, which could falsify the results. Since *Dirofilaria immitis* antigen was used in this study, formation of specific IgE antibodies to *Onchocerca volvulus* worm in these patients was not fully demonstrated as there may be antigenic differences. Furthermore, a better result could have been obtained using pooled control serum of Nigerian subjects, matched for age, sex, and tube, other than the pooled Californian American serum.

It is possible that there are other immunological factors involved in onchocerciasis. Histological granulomata occur in onchocerciasis (Harman, 1972) and it is possible that onchocerciasis, a chronic disease, may have both humoral and cell-mediated components.

One of the merits of the RAST, as described, is that it can be used as screening test for mass epidemiological surveys. This test can be carried out on the field using blood from finger pricks of onchocerciatric patients and blotting paper strips impregnated with *D. immitis* or *O. volvulus*, antigen.

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