Filarial complement-fixation test for pulmonary tropical eosinophilia with *Ascaris* antigen

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SYNOPSIS A trial of antigen prepared from *Ascaris lumbricoides* in the complement-fixation test for pulmonary tropical eosinophilia and for filariasis is described. No significant difference was observed between this antigen and that prepared from *Dirofilaria immitis*.

The disease which has come to be known as pulmonary tropical eosinophilia (Weingarten, 1943) has been commonly found in India and Malaya, though it has also been described elsewhere in the tropics. In its tropical (especially coastal) origin and in its failure to resolve spontaneously it differs from the similar condition known as Löffler's syndrome or eosinophilic lung. It affects in Britain only persons recently living in the tropics, and, above all, Indian seamen, among whom 37 cases have been diagnosed in this hospital during the last six years. Once discriminated from other forms of asthmatic bronchitis, pulmonary tropical eosinophilia may be cured by hetrazan, which has now replaced the original (also effective) intravenous arsenical treatment. It is thus an important disease to diagnose with accuracy, and this may be difficult with Indian seamen, whose histories are not adequate, whose eosinophilia may be due to intestinal helminths, and whose bronchi suffer from their change of climate. All inpatients are screened for eosinophilia by the use of special diluting fluid (Randolph and Stanton, 1945) in routine total white cell counts. The diagnosis then turns mainly on the association of eosinophilia which is high with asthmatic bronchitis. But confirmation by the filarial complement-fixation test has been found of great value. Originally Dr Ridley kindly performed these tests for us, using *Dirofilaria immitis* antigen (Ridley, 1956), then tests were tried here with extracts of the round worm *Ascaris lumbricoides* as being the nearest relation to the filarioids easily obtainable in large quantity.

MATERIALS AND METHODS

ANTIGEN Fresh *Ascaris lumbricoides* worms were washed, snipped into bits, and dried in a hot fan blast. Thorough

grinding with sand in ethanol (about 10 ml per worm) was followed by incubation at 37°C, shaking daily, for three weeks. After filtration through Whatman 40 paper, the fluid was stored at 4°C, refiltering if necessary. The working strength of various batches determined by chessboard titration with positive and negative sera and 2 MHD complement, was found to be about 1 in 250 and with 3 MHD complement was about 1 in 40.

TEST All four reagents (serum, antigen, complement, and sensitized cells) were used in 0.16 ml volumes. Sera were tested in rows of doubled dilutions from 1 in 5 to 1 in 320 in glass tubes, 11 mm diameter. Complement strength was 2 MHD. Incubation time was 60 min on the bench, then 60 min on a bath at 37°C before cells were added. The usual type of barbitone-buffered saline, with added Mg and Ca, was used as diluting fluid. Reactions of control sera from local antenatal clinics occasionally reached a dilution of 1 in 15, which was therefore taken as the critical level: readings were considered negative below 1 in 15 and positive above it. Finally, it was decided that test conditions employing about 1 in 40 antigen with 3 MHD of complement and a critical serum titre of 1 in 5 would have been slightly more convenient than the more dilute method actually used in all results with *Ascaris* antigen reported here.

RESULTS

Table I shows that of 202 tests the *Dirofilaria* antigen gave 149 negative (including 21 slight reactions) and 53 positive reactions, while the *Ascaris* antigen gave 156 negative and 46 positive reactions. Of the negative reactions, 137 coincided, though of the positive only 34 did so. Of nine cases of clinically diagnosed pulmonary tropical eosinophilia which were tested in parallel, all were positive with *Ascaris* but only seven with *Dirofilaria* antigen.

The smaller total number of positive results obtained with *Ascaris* compared with *Dirofilaria* antigen suggested that intestinal helminths were not

Received for publication 28 September 1967.

TABLE I

NUMBERS OF SERA REACTING IN COMPLEMENT-FIXATION TEST WITH *Dirofilaria* AND *Ascaris* ANTIGENS

	Dirofilaria Antigen			
	Negative	Slight Reaction	Positive	Total
<i>Ascaris</i> antigen				
Negative	121	16	19	156
Positive	7	5	34	46
Total	128	21	53	202

weighting the former figure. But when a larger series of results with *Ascaris* antigen was plotted against the presence or absence of faecal helminths (Table II), it appeared that a higher positive rate was associated with the presence of intestinal helminths (20.9% compared with 13.6%). This could be due to the fact that both filariasis and

TABLE II

NUMBERS OF PATIENTS TESTED BY *Ascaris* COMPLEMENT-FIXATION TEST AND FAECES EXAMINED FOR HELMINTHS

<i>Ascaris</i> Complement fixation Test	Intestinal Helminths		
	Absent	Present	Strongyloides Larvae
Negative	293	347	34
Positive	46	92	22
Positive (%)	13.6	20.9	39.3

intestinal helminthiasis tend to occur in the same environment. But a tendency was also noted for positive results to be associated with helminth fauna which included larvae of *Strongyloides stercoralis*. When such cases were extracted (Table II, column 3), it was seen that positive results had risen significantly to the level of 39.3%.

In Table III, results of the *Ascaris* complement-fixation test are plotted against clinical diagnosis, assigned to filarial, non-filarial, and pulmonary tropical eosinophilic categories. Patients were mainly natives of the tropics, whose sickness brought them to hospital, and it was therefore not possible to include a similar category of normals. The doubtful group contained 118 cases which could not be certainly placed in either filarial or non-filarial groups although it contained a good deal of suspected but unproved filariasis. To this group were also assigned cases of idiopathic eosinophilia coming from regions where filariasis is prevalent. Cases in which a moderate eosinophilia was accompanied by intestinal helminths (or other known cause) were assigned to the non-filarial group. The positive rate of 37.3% in the doubtful group, lies, as expected, between that of the filarial and non-filarial groups. Cases of hydrocele with eosinophilia, provided they came from the eastern side of the Indian continent,

TABLE III

CORRELATION OF *Ascaris* COMPLEMENT-FIXATION TEST WITH CLINICAL DIAGNOSIS

<i>Ascaris</i> Complement-fixation Test	Number of Cases Falling into Different Diagnostic Groups			
	Filarial	Non-filarial	Doubtful	Pulmonary Tropical Eosinophilia
Negative	5	705	74	2
Positive	12	63	44	35
Positive results as % total	70.6	8.2	37.3	94.6

gave 100% positive results. Three Guinea worm cases all gave strongly positive results. It will be seen that this hospital receives few cases of classical filariasis, but a relatively large number of cases of pulmonary tropical eosinophilia, for which Table III records 94.6% positive results, thus substantiating the usefulness of the test for its main purpose. Too many tropical patients recorded in Table III gave unsubstantiated positive results to warrant using the test in a similar diagnostic manner for filariasis as for pulmonary tropical eosinophilia.

Normal control sera for the results in Table III were provided by local antenatal clinics: of 1,113 such sera, eight (0.7%) were positive. The countries of origin of these eight mothers were Nigeria (4), Jamaica (2), Grenada (1), India (1): in other words, the test had probably selected seven filarial carriers from a thousand mothers in east London. In two of the Nigerians, microfilariae of *L. loa* were demonstrated. Europeans only gave false positive results in the very few cases when they were syphilitic, one being positive with *Dirofilaria* antigen also. The finding was checked by testing sera from VD clinics: those which reacted with *Ascaris* antigen were found to have titres of 1 in 50 or more in the cardiolipin Wassermann reaction. The reverse observation was also made: a positive cardiolipin Wassermann reaction was found to be associated with pulmonary tropical eosinophilia (as with glandular fever and malaria) in about a fifth of our cases, and to revert to normal after treatment. A high titre in the Reiter protein Wassermann reaction was not associated with a positive *Ascaris* complement-fixation test.

Since geography was evidently related to the *Ascaris* complement-fixation test results, patients have been roughly classified according to their native continent in Table IV, which shows a similar positive rate in the continents of India, Africa, and S. America with a lower one in the far East. Over three quarters of the cases are seen to have been Indians, and, as this group contains all the pul-

monary tropical eosinophilia, their true filarial rate would be somewhat lower than that of the African group.

TABLE IV
CORRELATION OF *Ascaris* COMPLEMENT-FIXATION TEST
WITH PLACE OF BIRTH

Place of Birth	No. of Cases	No. of Positive Reactions	Percentage Positive
Europe	117	2	1.7
Africa	89	19	21.3
India	703	137	19.5
Far East	67	4	6.0
Caribbean and S. America	34	6	17.6

DISCUSSION

The efficiency of the *Ascaris* complement-fixation test in the diagnosis of pulmonary tropical eosinophilia is shown by the figures in Table III, which also indicates that the test responds better to the antibody of this disease than to that of actual filarial infections, the titres being in fact much higher in the former as shown with *Dirofilaria* antigen by Danaraj, da Silva, and Schacher (1959). This is consistent with the allergic aetiology of the disease, experimentally confirmed by Buckley (1958) on a human volunteer. Microfilariae were absent from the blood in all our 37 cases.

One particular case (044268) in our general series was of special interest because of an association of pain over the liver, including an episode of colic, with high eosinophilia (3,600 per cu mm), strongly positive *Ascaris* and *Dirofilaria* complement-fixation tests, and striking failure to eradicate ascariasis by 11 courses of treatment (mainly Alcopar) over four months. Penetration of tissue by *Ascaris lumbricoides* was diagnosed after it was realized that *Ascaris*,

by the misplaced use of tetrachlorethylene, had probably been stimulated. In fact, it seems that *Ascaris* climbed the bile duct and may even have given rise to liver abscess as described by Reay, Dignan, and Maunder (1964). Apart from penetration of the tissues by *Ascaris* itself, *Strongyloides stercoralis* appeared the most antigenic intestinal parasite, and in one of our cases (054646), with one month's cough and chest pain, *Strongyloides* larvae in the faeces, normal sedimentation rate, 800 eosinophils per cu mm, and a rising titre in the *Ascaris* complement-fixation test, the differentiation from pulmonary tropical eosinophilia was only settled by the curative effect of dithiazanine. A small series of cases was also tested in parallel using antigen extracted from *Dracunculus medinensis* in the manner described above for *Ascaris lumbricoides*: the results, and even the titres, were the same as those with the *Ascaris* antigen.

The tentative conclusion of this work is therefore that a similar, and perhaps identical, nematode lipid is present in alcoholic extracts both of *Ascaris* and *Dirofilaria*, that it is antigenic if human tissue is penetrated, and that hyperimmunity may give cross reaction with cardioliipin antigen.

For a great deal of the recording, we are indebted to Mrs T. Sargeaunt.

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