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Immunological Diagnosis of Human Filariases: Present Possibilities, Difficulties and Limitations

(a Review)

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Contents

Abstract

The diagnosis of filarial infections is one of the most complex fields of parasitic sero-immunology. In this study the results of the last ten years are reviewed. The first part is an analysis of the most commonly used methods: skin test, complement fixation reaction, passive haemagglutination test, double gel diffusion, immuno-electrophoresis and indirect fluorescent antibody test.

The advantages and disadvantages of each of this methods are discussed. The most important problems of sero-immunological diagnosis of filariases are discussed, especially concerning the values of the uses of different specific antigens (i.e. *Wuchereria bancrofti, Onchocerca volvulus)* or group antigens *(Dirofilaria immitis* or *Dipetalonema viteae,* in general) as well as the availability of the antigens. The sero-immunological methods play an important role in the diagnosis of filariases, in post-therapeutic controls and in epidemiological and pathogenic research studies. The conclusions of different authors using the same methods may sometimes be contradictory and the interpretation of the results is often difficult because of cross-reactions between different nematodes.

1. Introduction

Filarial infections, which are widespread in several regions of the world, constitute a very heterogenous group of parasitic diseases. Some of them (wuchereriasis, onchocerciasis) may cause particularly severe complications.

Biological diagnosis of these diseases is normally carried out by examination for microfilariae, which are present in the blood or the dermal fluid. In spite of several recent technical improvements, this parasitological examination may be inadequate in cases of slight infection or because of the periodic nature and quantitative variations of the parasitaemia. From the practical viewpoint, this explains the importance attached to immunological diagnosis. From the theoretical viewpoint, research in this field is all the more justified because filariases develop over very long periods – most filariae parasitic to man live for 10 years or longer – and because these parasitic diseases are often accompanied by allergic manifestations that reflect the importance of the immunological phenomena they cause.

The immunological study of filariasis goes back almost 60 years, for the first work on the subject was published in 1916 (RODHAIN & VAN DEN BRANDEN). Since then there have been a very large number of publications on this subject and new ones appear each year. This wealth of literature reflects the importance of the question, and altogether it reveals definite technical advances. However, since the authors often use different immunological methods or antigenic reagents, the results sometimes seem contradictory, and it may be difficult for the reader to follow the development of ideas and to obtain a clear overall picture of what is one of the most complex fields of parasitic sero-immunology.

Here therefore we shall attempt to present a general review. It is based essentially on the most important publications of recent years. A remarkably full general survey of older works was published by KAGAN in 1963.

First of all we shall consider the various methods: the examination for skin hypersensitivity and *in vitro* serological tests. In each case the references and the chief results are presented in tables, then discussed. In a later section the essential features of these analytical reviews are grouped together so as to reveal the present possibilities, the difficulties and the limitations of the immunological diagnosis of filariasis.

2. The Methods and their Results

2.1 Examination for skin hypersensitivity (skin test)

This is by far the most frequently used method: even in 1963 KAGAN listed 79 publications on the skin test. Table 1 summarizes a few of the main results obtained since that time.

Table 1. Examination for skin hypersensitivity (skin test)

Authors	Antigens used	Country and filariases studied	Results: No. of positive cases/ No. of cases examined		Observations
HIGASHI, DASTIDAR & CHOWDHURY (1968)	Saline extracts:	JAPAN			Negative controls
	W.b. μF	Wuch.	F+ 5/10	Eleph.	19+/21
	W.b. i.l.		F+ 13/16	13/15	4+/17
	D.i. Ad.		F+ 16/16	15/17	31+/38
	Purified antigen			4/4	Larval antigen the most
	FSCD 1				specific
SAWADA, SATO, MATSUYAMA, MIYAGI & SHINZATO (1968)	D.i. Ad.	JAPAN			In endemic zone: 340/403:
	Purified antigen	Wuch.	F+ 80/91	(88 %)	increased percentage with
	FSCD 1		F– S+ 15/20	(75 %)	age between 15 and 24 years:
			Treated controls:		96.4 % reactions +
			65/85	(77 %)	5.5 % microfilaraemia +
GIDEL, BRENGUES & RODHAIN (1969)	D.i. Ad.	UPPER VOLTA			F– and S– subjects: 56/102
	Purified antigen	Onch.	F+ 15/20	(75 %)	(54.9 %). In endemic zone:
	FSCD 1	Wuch.	F+ 22/29	(75.9 %)	no difference by sex; in-
		D. perst.	F+ 44/52	(84.6 %)	creased percentage of reac-
		Wuch. + Onch.	F+ 3/4		tions with age in F– subjects
		Wuch. + D.p.	F+ 38/51	(74.5 %)	
		Onch. + D.p.	F+ 38/69	(55 %)	
		O. + W. + D.p.	F+ 31/42	(73.7 %)	
SAWADA, SATO & SATO (1969)	D.i. Ad.	JAPAN			
	Various fractions obtained by chromatography	Wuch.			
	FST 3		F+ 24/26		
	FST 5		F+ 22/26		
	FST 1		F+ 7/26		
	FST 2		F+ 4/26		
	FST 4		variable reactions		
	After electrophoresis, fract.				
	FST 3 1		F+ 14/18		
	FST 2 x		F+ 16/20		
ULRICH, PINARDI & CONVIT (1970)	O.v. μF	Onch.	F+ 53/61	(86 %)	Negative controls: 4+/13
SMITH, WILSON, BEREZANCEV, LYKOV, MYO PAING, SAWADA, CHARI & DAVIS (1971)	D.i.	BURMA			+ reactions with solvents:
	Purified antigen	Wuch.	F+ 83/90		11/90; 10/30; 7/47 in the 3
		INDIA			previous groups.
		Wuch.	F+ 29/30		Burmese children with in-
		TANZANIA			testinal verminoses: 48+/60
		Wuch.	F+ 40/47		(16+ with solvent).
					Liverpool control group:
					11+/61 (4+ with solvent).
					Leningrad control group:
					13+/55 (1+ with solvent)

2.1.1 Antigens used

For a long time the antigens were prepared from very different parasites, and altogether at least 14 species of filariae have been used. Since 1965, following the work of SAWADA et al., there has been a tendency mainly to use purified extracts of adult *Dirofilaria immitis*. These extracts have been precisely analysed (SAWADA, SATO & SATO, 1969): using chromatography with DEAE-sephadex, followed by electrophoresis, it was possible to isolate a fraction made up of a single protein and presumed to be the most active fraction (FST 3 1).

2.1.2 Reading conditions

Most authors regard the test as positive if a wheal at least 0.4 cm^2 in area (7 mm in diameter) is observed 15 minutes after intradermal injection of 0.2 ml of antigen. Agreement is far from complete, however, and as SMITH et al. (1971) pointed out the extreme limits of readings accepted as positive vary widely: CIFFERI et al. (1965) accept an induration of 1 cm^2 (11 mm in diameter), while KATAMINE (1969) accepts an increase of 3 mm in the diameter of the wheal over its initial value. Applying these criteria of positive reactions in turn, SMITH et al. (1971) obtained, respectively, 22 % of false positive reactions in control subjects and 53–85 % negative reactions among confirmed filariasis cases.

2.1.3 Specificity

This is clearly dependent on the antigen and on the conditions under which the test is read. Even using *D. immitis* antigen, positive tests with wheals of 0.4 cm^2 are often observed in non-filarial subjects. Outside the endemic areas the incidence of these cross-reactions varies between 26 % and 38 % of cases (SMITH). In tropical countries it reaches 54.9 % (study by GIDEL et al., 1969, using Sawada antigen) and even 80 % according to SMITH et al. (1971). According to these authors, false positive reactions can be observed in 10–30 % of cases when the solvent alone is injected.

Moreover, there are many cross-reactions between the various human filariases and the Sawada *D. immitis* antigen cannot be regarded as specific to wuchereriasis.

Abbreviations

B.m. = *Brugia malayi*
D.i. = *Dirofilaria immitis*
D.m. = *Dracunculus medinensis*
D.r. = *Dirofilaria repens*
D.sp. = *Dipetalonema sp.*
D.v. = *Dipetalonema viteae* (or *witei*)
L.c. = *Litomosoïdes carinii*
L.l. = *Loa loa*
O.v. = *Onchocerca volvulus*
S.c. = *Setaria cervi*
S.l. = *Setaria labiatopapillosa*
W.b. = *Wuchereria bancrofti*
A.s. = *Ascaris suum*
F.h. = *Fasciola hepatica*
S.m. = *Schistosoma mansoni*

μF = microfilariae
i.l. = infesting larvae
Ad. = adults
F+, F– = presence or absence of microfilariae
S+, S– = presence or absence of clinical signs of filariases
Onch. = Onchocerciasis
Wuch. = Wuchereriasis
Drac. = Dracunculiasis
D. perst. = *Dipetalonema perstans* filariasis
D. rep. = *Dirofilaria repens* filariasis
D. imm. = *Dirofilaria immitis* filariasis
D. sp. = *Dirofilaria* sp. filariasis

In the study by GIDEL et al. (1969), in fact, the highest percentage of positive reactions was obtained for infection with *Dipetalonema perstans* (84.6 %), while onchocerciasis gave almost as many positive reactions (75 %) as wuchereriasis (75.9 %).

2.1.4 Reproducibility

This received a severe assessment from SMITH et al. (1971), who repeated the test on 56 subjects and obtained results ranging for example from 0.7 to 1 cm^2. Clearly, this low reproducibility makes it even more difficult to establish a criterion of positive reactions.

2.1.5 Diagnostic and epidemiological importance

The skin test is easy to use in the field and is entirely suitable for epidemiological surveys, providing much more faithful results than examination for microfilariae (surveys on wuchereriasis by SAWADA et al., 1968). In the endemic zone the positive reaction rate increases with the age of the population examined.

2.2 *In vitro* tests for the study of cell-type immunity

These tests are still very little used in filariasis. However, the study by PINON & GENTILINI (1973) shows the importance of the leukocyte migration inhibition test and the rosette test. When carried out with an antigen extracted from *Onchocerca volvulus* these tests appear to be specific. They were consistently positive in 15 patients suffering from various filarial diseases who sometimes yielded negative results in serological tests. Since they are relatively complicated, these tests seem primarily suitable for basic research, particularly as the precise nature of the phenomena they reveal remains debatable.

2.3 Complement fixation reaction (Table 2)

This is the serological test with the longest history of application to filariasis (RODHAIN & VAN DEN BRANDEN, 1916). For a long time it was the most frequently used (44 references given by KAGAN in 1963). In recent years workers seem to have attached less importance to it, and the chief recent study is that by GIDEL et al. (1969) using the Sawada antigen. Positive reaction rates varying from 10–60 % were obtained for the various types of filariasis. Paradoxically the Sawada antigen, when used in the skin test in the same study, gave 75 % positive reactions for onchocerciasis and 75.9 % for wuchereriasis, but when used in the complement fixation test it produced 60 % positive reactions for onchocerciasis and only 10.4 % for wuchereriasis. This discrepancy might be associated with poor preservation of some sera, with poor anticomplementary potency, or with the fact that in microfilaraemic filariasis the microfilariae may neutralize the circulating antibodies.

From the epidemiological viewpoint, moreover, the skin test is more reliable than the complement fixation test. The latter reveals no significant difference between subjects with microfilaraemia and others (which tends to invalidate the hypothesis given above), but its positive reaction rate rises very substantially with the age of the patients examined.

Table 2. Complement fixation reaction (CF)

Authors	Antigens used	Country and filariases studied	Results: No. of positive cases/ No. of cases examined	Observations
LARTIGUE (1965)	O.v.	Onch.	F+ 39/49 (78 %) F– S+ 13/20	
GIDEL, BRENGUES & RODHAIN (1969)	D.i. (Sawada antigen)	UPPER VOLTA		Sero-epidemiological survey:
		Onch.	F+ 12/20 (60 %)	F+ 34.5 % pos. in CF
		Wuch.	F+ 3/29 (10.4 %)	F– 38.2 % pos. in CF
		D. perst.	F+ 14/52 (26.9 %)	difference not significant
		Wuch. + Onch.	F+ 1/4	
		Wuch. + D.P.	F+ 9/51 (17.7 %)	No difference by sex
		Onch. + D.P.	F+ 39/69 (56.5 %)	
		Onch. + D.P. + Wuch	F+ 14/42 (33.3 %)	Marked increase in positive reaction rate with age
TANAKA, FUJITA, SASA, TAGAWA, NAITO & KURIKAWA (1970)	D.i. L.c. S.c.	Wuch. and experimental filariases due to L.c., S.c., B.m., D.v.		Study of different extraction procedures Best results obtained with a coca solution

* *Abbreviations:* see Table 1.

2.4 Passive haemagglutination tests (Table 3)

Studies published since 1963 show that on the whole this test gives fairly reliable positive results for the various types of filariasis. Unfortunately its specificity appears to be low, since cross-reactions may be observed with certain feverish conditions or with syphilis (ROSÉ et al., 1966), with about 10 % of the various parasitic diseases (KAGAN et al., 1963), and indeed with almost 35 % of trichinoses. Conversely, antigens extracted from *Ascaris suum* yield positive results with sera from filarial subjects in 30–60 % of cases (ROSÉ et al., 1966).

2.5 Precipitation tests: double gel diffusion and immuno-electrophoresis (Table 4)

Although precipitation tests in liquid medium are hardly used any longer, double gel diffusion and immuno-electrophoresis are attracting increasing interest in the study of filariasis. Methodologically, the Ouchterlony test is generally used as a screening test as a kind of preliminary to immuno-electrophoresis. Under simple conditions and comparatively quickly, this test permits the filariases to be distinguished from other helminthic tissue infections capable of causing eosinophilia. It takes several days to carry out immuno-electrophoresis but the specificity of the test at present seems unsurpassable: in loaiasis, wuchereriasis and onchocerciasis, characteristic precipitation arcs are obtained not only with the homo-

Table 3. Passive haemagglutination reaction (HA)

Authors	Antigens used	Country and filariases studied	Results: No. of positive cases/ No. of cases examined	Observations
KAGAN, NORMAN & ALLAIN (1963)	D.i.	USA	F+ 12/13	10 % cross-reactions with various parasitic diseases
		D. perst.	F+ 8/23 (Agglutination test with sensitized bentonite particles)	35 % with trichinosis
ROSÉ, BIGUET, ROSÉ & D'HAUSSY (1966)	O.v.	FRANCE		Weak positive reactions for feverish infections or syphilis
		Onch.	F+ 23/23	
		D. perst.	F+ 5/5	
		Drac.	F+ 9/9	
		Loaiasis	F+ 2/2	
		Wuch.	F+ 2/2	
	A.s. Antigen extracted from whole worm	Onch.	F+ 3/11	*Ascaris* antigen: limit of specificity of test at least 1/128
	Coelomic fluid		F+ 6/11	
FUJITA, TANAKA, SASA, SHICHINOHE, ASAI & KUROKAWA (1970)	L.c. D.v. B.m. S.c. D.v.	JAPAN Experimental filariases due to D.i., L.c., S.c., and D.v.		
PINON & GENTILINI, (1973); GENTILINI, PINON, NIEL & ROSIN (1973)	O.v.	FRANCE		10 health subjects and 10 patients with schistosomiasis or intestinal anguilluliasis: all negative
		Wuch.	F+ 0/3	
		Onch.	F+ 4/8	
		Loaiasis	F+ 1/2	
		Drac.	F+ 1/1	

* *Abbreviations:* see Table 1.

logous antigens, but also with extracts from *D. witei* (CAPRON et al., 1968) or *A. suum* (GENTILINI et al., 1972a). This paradoxical finding is explained by the very analytical character of the method, which can reveal antigenic fractions common to *Loa loa, Wuchereria bancrofti* or *O. volvulus* and *D. witei* or *A. suum*. These common fractions vary from case to case, so that one heterologous antigen ultimately leads to specific results. Although using a completely different methodological approach, the Japanese school of Sawada is really working on the same problem; on the basis of extracts from *D. immitis,* it is attempting to isolate as specific a *W. bancrofti* fraction as possible.

In sero-epidemiological surveys, immuno-electrophoresis faithfully reflects the level of onchocerciasis endemicity in the regions studied (D'HAUSSY et al., 1972). However, it is not certain that this method, which requires large quantities of both antigen and sera, is best applied for this purpose.

From the diagnostic viewpoint this test provides positivity rates that are slightly lower than those provided by other methods (immunofluorescence). On

Table 4. Double gel diffusion precipitation tests, Ouchterlony test (OT): immuno-electrophoresis (IEP)

Authors	Antigens used	Country and filariasis studied	Results: No. of positive cases/ No. of cases examined	Observations
BIGUET, D'HAUSSY, AUBRY & ROSÉ (1964)	O.v. D.v. D.i. A.s. S.m. F.h.	FRANCE Onch.	F+ 222/250 (88.7 %) F+ 10/11 F+ 5/6 F+ 23/30 F+ 2/16 F+ 7/24	Tests used: OT and IEP Cross-reactions with O.v. antigen: Healthy subjects: 2/37; Drac.: 1/28; D. perst.: 2/9; Wuch.: 0.2; Loaiasis: 0/2; various parasitic diseases: 5/34
DODIN, MOREAU & LAMBERT (1965)	D.v. – μF – Ad.	FRANCE Wuch.	F+ 3/5 F+ 1/5	OT only
ROSÉ, BIGUET, ROSÉ & D'HAUSSY (1966)	O.v.	FRANCE Onch.	F+ 3/23	OT and IEP
CAPRON, GENTILINI & VERNES (1968)	D.v. O.v. D.i.	FRANCE Onch. Loaiasis Wuch. Drac.	87/100, with 88 F+ F+ 7/16 F– 12/13 F+ 3/10 F– 18/18 5/9 with 8 F+	OT and IEP D.v. and O.v. antigens give comparable results for Onch. D.v. antigen gives major arcs specific to loaiasis, Onch. or Wuch. higher percentage of positives in F– subjects than in F+ subjects
ULRICH, PINARDI & CONVIT (1970)	O.v. – Ad. – μF	Onch.	F+ 32/50 F+ 3/50	OT only
D'HAUSSY, CAPRON, ROLLAND & BIGUET (1972)	O.v.	FRANCE Onch.	– 94/97 (hyperendemic zone) – 99/133 (mesoendemic zone) – 12/27 (hypoendemic zone)	OT and IEP Sero-epidemiological survey in Upper Volta: 500 subjects examined
GENTILINI, PINON, NIEL & DANIS (1972b)	A.s. Coelomic fluid (A.s., c.f. antigen) Genital tract extract (A.s., O.g. antigen)	FRANCE Onch. Loaiasis Wuch. Drac.	F+ 130/132 (98.5 %) F+ 64/68 (94 %) F+ 19/30 (63.3 %) F+ 60/72 (83.5 %)	OT and IEP Study of total of 530 patients, of whom 228 F–
NIEL, GENTILINI, COUTURE, PINON & DANIS (1972)	O.v. Extracted from adult filariae, nodule walls or whole nodules A.s. (A.s., c.f. or O.g. antigen) D.v., Ad.	FRANCE Onch. Loaiasis Wuch. Drac. Loaiasis Wuch. Drac.	F+ 104/108 (96.3 %) F+ 105/108 (97.3 %) F+ 28/30 (93.3 %) F+ 19/20 (95 %) F+ 12/12 F+ 28/30 (93.3 %) F+ 9/12 (75 %) F+ 8/8	OT only

Table 4 (continued)

Authors	Antigens used	Country and filariasis studied	Results: No. of positive cases/ No. of cases examined	Observations
PETITHORY, BRUMPT, JAEGER & SOILLEUX (1972)	i.l., μF	FRANCE Loaiasis Wuch. Onch. Drac. D. perst.	 F+ 40/54 (74 %) F+ 3/19 (19 %) F+ 14/24 (58 %) F+ 0/7 F+ 3/27 (11 %)	OT only Specificity checks: 25 healthy subjects neg., 2 pos. results in 12 subjects with visceral larva migrans

* *Abbreviations:* see Table 1.

the other hand, its remarkable specificity makes it possible not merely to give a "general" diagnosis but to specify the type of filariasis.

Immuno-electrophoresis is the test in which positivity rates for patients with and without detectable microfilaraemia show the greatest difference.

Finally, developments after treatment are by no means identical, and there may be either a temporary increase or an immediate drop in the number of precipitation arcs.

Quite recently the electro-immunodiffusion test was applied to various parasitic diseases, particularly the filariases (GENTILINI et al., 1972a). The principle of this test, carried out on cellulose acetate membrane, is analogous to that of double gel diffusion, but the migration of the serum antibodies and the parasitic antigen is greatly speeded up by the action of a difference in potential at the terminals of the plate. Judging by the preliminary results at present available, this test could be of very great interest, since its diagnostic value seems almost comparable to that of immuno-electrophoresis and it can be carried out much faster.

2.6 Indirect fluorescent antibody tests (Table 5)

Starting from a single principle, three different types of test have been developed: they are considered in turn below.

2.6.1 Test using soluble antigen (SAFA test)

The antigenic reagent consists of discs of special paper impregnated with soluble filarial antigens. Initially, therefore, this test is subject to all the difficulties involved in extracting and purifying such antigens. On the other hand, the method is suitable for objective reading with a fluorimeter and, above all, it can be automated, which means that it could be used in large-scale epidemiological surveys. The present results seem promising, but they are still too few in number for any definite assessment to be made of the diagnostic value and in particular of the specificity of the test.

2.6.2 Test using microfilariae or whole or fragmented larvae

In general, whole microfilariae fix fluorescent antibodies only poorly, and fragments obtained by crushing or ultrasonic disintegration yield much better

Table 5. Indirect fluorescent antibody test

Authors	Antigens used	Country and filariases studied	Results: No. of positive cases/ No. of cases examined	Observations
		I. Test using soluble antigen (SAFA test)		
DUXBURY & SADUN (1967)	D.m. D.i. D.v.	USA Wuch. Onch.	212/259	
COLWELL, ARMSTRONG, BROWN, DUXBURY, SADUN & LEGTERS (1969)	D.i.	USA Wuch.	F+ 16/19 (84.2 %) F– 70/137 (51 %) (subjects born in endemic zone)	Sero-epidemiological survey in Viet-Nam, among US soldiers: 17/112 (15.2 %) in advanced positions and 5/91 (5.5 %) at base outside the operational zone
		II. Tests with microfilariae or with whole or fragmented larvae		
CHOWDHURY & SCHILLER (1962)	W.b. B.m. whole μF	USA Wuch. B.mal.	F+ 9/9 F+ 1/1	
LUCASSE (1962) LUCASSE & HOEPPLI (1963)	O.v. whole μF	Onch.	F+ 50/50 (8 of them weakly)	
MANTOVANI & SULZER (1967)	D.i. D.r. D.sp. – fragm. μF	USA D. imm. D. rep.	F+ 8/8 F+ 12/12	
MULLER (1970)	D.m. first-stage larvae	Drac.	F+ 33/34	Cross-reactions with Onch.
YONG (1973)	W.b. μF and first-stage larvae	FIJI Wuch.	with elephantiasis 60/60	No cross-reactions with 105 control sera
		III. Tests on frozen sections of adult filariae		
COUDERT, AMBROISE-THOMAS, KIEN TRUONG & TERRENO (1968)	D.i. D.v.	FRANCE Wuch. D. perst. Loaiasis Onch. Drac.	 F+ 1/2 F+ 6/8 F+ 38/38 F+ 11/11 F+ 3/4	D.i. and D.v. antigens equivalent
AMBROISE-THOMAS (1969)	D.i. D.v.	FRANCE Wuch. D. perst. Loaiasis Onch. Drac.	 F+ 2/3 F+ 7/9 F+ 48/51 F+ 12/12 F+ 4/4	Specificity checks on 200 control sera, specificity threshold 1/20. Progression after treatment variable

* *Abbreviations:* see Table 1.

Table 5 (continued)

Authors	Antigens used	Country and filariases studied	Results: No. of positive cases/ No. of cases examined	Observations
TERRENO (1970)	D.i.	FRANCE	F+ 5/5	Loaiasis F– 65/75 (87 %)
	D.v.	Drac.	F+ 21/25 (80 %)	Wuch. F– 7/19
		D. perst.	F+ 88/97 (90 %)	Onch. F– 16/20 (80 %)
		Loaiasis	F+ 8/10	Sero-epidemiological survey
		Wuch.	F+ 25/27 (92 %)	on Onch., 258 sera: results
		Onch.	F+ 7/7	parallel to level of endemi-
		Loaiasis + Onch.		city
GENTILINI, PINON, NIEL & DANIS (1972b)	D.i. S.l.	FRANCE Onch.	F+ 196/234 (84.2 %) F+ 73/90 (80 %)	Specificity threshold applied: 1/100
		Loaiasis	F+ 31/48 (61 %)	
		Wuch.	F+ 57/72 (77 %)	
		Drac.	F+ 74/86 (84 %)	
		Unidentified filariases		
GENTILINI (1973) PINON, RAFFIER & NIEL (1972c)	D.v. S.l.	FRANCE Drac.	F+ 306/356 (85.96 %) F+ 296/356 (83.16 %)	Specificity checks on 100 sera (various parasitoses): all negative at dilution threshold of 1/80
AMBROISE-THOMAS & KIEN TRUONG (1972)	D.i. D.v.	FRANCE Drac.	F+ 6/6	980 specificity checks. 934 Europeans: 98.2 % negative at 1/20. 254 Africans: 88.8 % negative at 1/20
		Wuch.	F+ 88/103 (85.4 %)	
			F– 89/96 (91.9 %)	
		Loaiasis	F+ 158/180 (87.8 %)	
			F– 170/187 (90.9 %)	
		Onch.	F+ 179/197 (94.7 %)	Inverse checks: 67 filarial
			F– 64/69 (92.7 %)	sera tested against *S. mansoni, F. hepatica, E. granulosus, A. lumbricoides, S. stercoralis* and *T. gondii*
		Loaiasis + Onch.	F+ 21/22 (94.5 %)	
		Loaiasis + D. perst.	F+ 24/26 (92.3 %)	
		D. perst.	F+ 95/113 (84 %)	
	L.l.	Loaiasis	F+ 14/14 (average titre 125)	Progression after treatment very variable
	D.v.	Loaiasis	F+ 14/14 (A.T. 56)	
	W.b.	Wuch.	F+ 29/31 (93.5 %, A.T. 47.1)	
	D.v.	Wuch.	F+ 27/31 (83.1 %, A.T. 27.7)	
	O.v.	Onch.	F+ 52/52 (A.T. 222)	
	D.v.	Onch.	F+ 51/52 (A.T. 145)	
DIESFELD & BRAUN-MUNZINGER (1972)	D.v.	GERMANY Wuch.	62/106 (F+ and F–)	Study mainly intended to check the accuracy of micro-sampling
PINON & GENTILINI (1973)	O.v.	Wuch.	F+ 1/3	
GENTILINI, PINON, NIEL & ROSIN (1973)		Onch.	F+ 7/8	
		Loaiasis	F+ 2/2	
		Drac.	F+ 1/1	

* *Abbreviations:* see Table 1.

results. Moreover, this type of corpuscular antigen adheres poorly to object slides. The test is therefore generally carried out in tubes, which reduces its convenience. It appears to give high positivity rates, but its specificity has not yet been adequately checked and in any case seems to lead to divergent conclusions.

2.6.3 Test on sections of adult filariae

From the technical viewpoint, this method has the drawback of requiring special and comparatively expensive equipment for preparing the antigens (taking sections of frozen specimens). On the other hand, one adult filaria provides sufficient antigenic reagents for several thousand tests; these antigens can readily be fixed on microscope slides and obviously cause none of the difficulties of extraction and purification that are associated with soluble antigens. The test can be carried out by counter-staining with Evans blue (Ambroise-Thomas) or without a contrast stain (Gentilini). In this case non-specific fluorescence may hamper reading of the results and, despite technical precautions such as the use of a Teepol solution for rinsing (PINON & GENTILINI, 1972), the specificity threshold rises from 1/20 to 1/80 or 1/100.

This test, which is easy to perform, has been extensively used in recent years. Its specificity (checked on a total of more than 2,000 sera) appears satisfactory.

From the diagnostic viewpoint this at present appears to be the most reliable test, with positivity rates between 85 % and 90 % (in subjects without detectable microfilaraemia, positive fluorescent antibody reactions are more frequent and more marked than in other subjects, but this difference is less marked than in immuno-electrophoresis). However, there are very considerable cross-reactions between the various types of filariasis, which it is impossible to distinguish by this method, using a group antigen such as *D. witei.*

To some extent the simultaneous use of *Loa loa, W. bancrofti* and *O. volvulus* antigens permits a more precise diagnosis by revealing varying increases in fluorescent antibody titres.

The movement of the antibody titres after treatment is very variable: there may be either a temporary rise or an immediate decrease.

This test, which requires little antigenic material and can be carried out with microsamples of blood, is very suitable for sero-epidemiological surveys (DIESFELD & BRAUN-MUNZINGER, 1972); several such surveys are in progress in various endemic regions.

3. General Discussion

Basically, the immunology of filariasis leads to consideration of the main technical aspects of the available tests and of the results provided by those tests in regard to the diagnosis, the post-treatment surveillance and the epidemiological study of filarial infections.

3.1 Technical problems

3.1.1 Filarial antigens used

For the filariases, more than for most other parasitic diseases, the quality of the antigenic reagents is the fundamental factor in any immunological study. These antigens present two problems, one con-

cerning the parasitic material from which they are extracted, the other concerning their preparation and purification.

(a) *Origin.* Except perhaps for *O. volvulus,* it is practically impossible to obtain sufficient filariae of the species parasitic to man to extract antigenic reagents. This leads either to the use of other nemathelminths such as *Ascaris suum* (studies by BIGUET et al., 1965, and by GENTILINI et al., 1972a) or, which is certainly preferable, to the use of filariae that are parasitic to various animals. At present, *D. immitis* is usually selected. However, this species is becoming increasingly difficult to obtain in a number of countries where *D. witei* – whose cycle is readily reproduced in the laboratory – is used as a source of antigen. It seems that these two species provide largely comparable results, but checks are still essential, particularly as regards the value of the *D. witei* antigen in intradermal tests.

As regards the stage of development of the parasite, most authors prepare their antigenic extracts from adult filariae. This seems perfectly justified, particularly for practical reasons. Nevertheless, it should be checked that the larval stages (microfilariae or infesting larvae collected from the arthropod intermediate host) have no special antigenicity, as some studies appear to show.

(b) *Preparation and purification.* Two types of antigenic reagent can be used: "corpuscular" antigens (larval stages, fragments or sections of filariae) and "soluble" antigens extracted from the parasites.

In the former case, the preparation conditions are generally simple and there are no problems of purification. These antigens can be used only *in vitro* and for one test (indirect fluorescent antibody test); they appear to give fairly satisfactory results, although these results cannot be strictly specific to a particular filariasis.

The soluble antigens are generally obtained by freeze-drying the worms, crushing them and removing the lipids, followed by the action of a saline solution. The principal difficulty lies in their purification, for the filariae have a complex antigenic constitution and within this "antigenic mosaic" there are a very large number of fractions that are common to all filarial species and are even found in most other nemathelminths. In one way these common antigenic features are an advantage, since they make it possible to prepare antigens from filariae parasitic to animals. On the other hand, they clearly make it very complicated to purify the reagents afterwards.

The dilemma, therefore, is that a non-purified antigen is certainly not specific, while on the other hand there is the risk that a specific fraction oftained by excessive purification may no longer be active. This shows how essential is the research in this field being conducted by several teams (SAWADA). At each stage of the experiment, however, it would be desirable to carry out more numerous trials so as to permit

a better assessment of the value of the various fractions isolated and a comparison with the value of antigens obtained by more simple means. At present, these comparative checks generally cover only a few dozen tests, which is not sufficient to justify definite conclusions.

3.1.2 Performance of the tests and interpretation of the results

The standardization of immunological tests is still a source of many difficulties. In the intradermal test such standardization seems almost to have been achieved (inject 0.2 ml of antigen, read after 15 minutes by measuring the area, not the diameter, of the wheal, which should be at least 0.4 cm^2 for the test to be positive). However, it still remains to define the characteristics of the antigen more precisely, both quantitatively (concentration of total nitrogen) and qualitatively (e.g. checks using immuno-electrophoresis).

In the case of serological tests, it is probably pointless at present to hope for complete uniformity of operating conditions. Workers could at least follow the example of what has been done for toxoplasmosis – incidentally at the instigation of WHO – and adopt the system of pools of reference sera, so that results could be expressed in International Units. This could easily be done and would have the immense advantage of making all the results of quantitative tests (complement fixation, passive haemagglutination, agglutination, fluorescent antibody) mutually comparable, which is not permitted by the present method of recording results (in terms of dilution rates) that does not refer to any common standard.

3.2 Results

3.2.1 Immunology in the diagnosis of filariasis

A very large number of cases have been studied by the various immunological methods. On the whole, these tests seem considerably more reliable than the traditional procedures of examination for microfilariae. This comparison should be reconsidered, taking into account the recent technical improvements in the parasitological diagnosis of filariasis (e.g. use of microfiltration). Moreover, it is a pity that the different serological tests cannot be rigorously compared: most studies are carried out by a single method, and it is unusual for the same patients to be examined by different tests applied in parallel.

As regards diagnosis, the main problem presented by immunological tests concerns the specificity of the results. This problem must be considered at three different levels.

First, some non-specific reactions are associated with the method itself (positive intradermal reactions following injection of the solvent

alone). They involve special cases only (hyperergic patients) and seem difficult to avoid.

The second aspect concerns cross-reactions between filarial infections and other diseases, whether parasitic or not. In the present state of techniques and reagents, these false positive reactions appear to be numerous (2–30 %, depending on the test) and mainly concern the various roundworm helminthiases. Efforts must clearly be made to reduce this risk of error, and also to make a better evaluation of its current level. In particular, cross-reactions are much more frequent in tropical countries where many nematodoses are prevalent. In such countries, therefore, more extensive control of the specificity of immunological tests is particularly important. At the same time this is very difficult to achieve, in that the study must be carried out on subjects who are definitely not infected with filariae but who live in areas where various filariases are often endemic.

Finally, in so far as the other problems of specificity are solved, immunological tests generally supply only a general diagnosis, and the extent of the common antigenic features shared by the filariae almost always prevents workers from taking the diagnosis further and distinguishing between the various filariases. Such a distinction is all the more important because these diseases are of varying severity, and treatment with diethyl-carbamazine causes side effects that are not justified when the filariae present are non-pathogenic (e.g. *D. perstans*); at present a precise diagnosis can be established only by combining several methods. The immunological tests may be regarded primarily as a screening method; if they should prove positive, numerous parasitological examinations may be conducted until the microfilariae are discovered and identified. It is also possible to apply several immunological tests whose results complement each other – intradermal test, complement fixation, passive haemagglutination, double gel diffusion, or fluorescent antibody – as screening tests (general diagnosis of "filariasis"), followed by immuno-electrophoresis for the precise identification of the filarial species (specific precipitation arcs). With indirect fluorescent antibody tests, simultaneous use of several specific antigens may also lead to the same result. In many cases, however, serological ambiguity persists (absence of specific arcs in immuno-electrophoresis; similar fluorescent antibody titres with all antigens) and it is impossible to tell whether it is the method that is at fault or whether the patient is harbouring several types of filariae simultaneously.

3.2.2 Immunology in the post-therapeutic control of filariasis

Serological developments after treatment are not uniform. Sometimes there is a temporary rise in antibody titres or in the number of precipitation arcs, sometimes an immediate drop. These variations are

no doubt due to the fact that diethylcarbamazine, which gives rise to side effects, is usually prescribed in very gradually increasing doses and is administered at the same time as various antihistamines or corticoids. In any case, this field has still been inadequately studied. In particular, it is not known whether, after a complete cure, the serological findings become completely negative, and if so within what period. This information would be particularly valuable from the practical viewpoint since it is difficult to make a definite verification of a cure by other biological tests.

3.2.3 Immunology in the epidemiological study of filariasis

During the last 10 years, immunological techniques have on several occasions been used in epidemiological studies. On the whole the results appear conclusive, particularly in the case of wuchereriasis where the parasitological diagnosis encounters various practical difficulties regarding sampling "in the field". However, some of these surveys are "sero-epidemiological" only in name and mainly amount to a sero-immunological study grafted more or less artificially on to epidemiological surveillance campaigns. It would therefore be desirable to resume and expand studies of this kind but in a different spirit, regarding immunology as a means and not as an end in itself. In assessing the results, not only the accuracy of the results but also the convenience of using the methods should be taken into account. In addition, at least some of these studies should be conducted by a longitudinal rather than a pinpoint technique. This would permit a better assessment of the methods' epidemiological value, and in particular their reproducibility. In the case of intradermal tests it would be essential to know whether there is a risk that repeated intradermal injections may lead to positive reactions to skin tests or serological tests.

3.2.4 Immunology in the biological or pathogenic study of filariasis

Sero-immunological tests, which reflect the extent and complexity of host-parasite relationships, are particularly suitable for certain basic research.

Among the many problems, it would be interesting to know more about the relationships between serology and microfilaraemia and between serology and clinical severity in the various filariases.

As regards the former relationship, it seems that in infections with sanguicolous microfilariae, serological tests are more often positive in subjects with negative microfilaraemia than in other patients. However, there is no unanimous agreement on whether this phenomenon actually occurs (e.g. GIDEL et al., 1969, found no significant difference in the complement fixation reaction) or on its extent (the differences appear much greater in immuno-electrophoresis – CAPRON et al., 1968, 1970 –

than in the fluorescent antibody test – AMBROISE-THOMAS & KIEN TRUONG, 1972). More thorough research on the subject is therefore essential. In particular, it is important to define clearly the criteria whereby a patient without microfilaraemia may still be regarded as filarial. It will then still be necessary to explain the mechanism whereby microfilaraemia may modify the circulating antibody titres. The hypothesis most frequently put forward involves a blocking process *in vivo* (whereby the microfilariae fix the antibodies, which, when there is very little or no microfilaraemia, remain free and therefore readily detectable), but it needs to be confirmed.

As regards the pathogenesis, it is possible that the sero-immunological tests will contribute to the recognition and better understanding of the severity of certain symptoms and various complications. In a preliminary study, for example, this has been achieved for the diagnosis of filarial arthritic conditions by means of the rosette test (WALTZING & BLOCH-MICHEL, 1971). For wuchereriasis, likewise, it seems that patients with elephantiasis have a particularly high immunological response. In another field, it is known that onchocerciasis in West Africa causes more blindness in savanna regions than in forest regions. The serological tests might perhaps permit a more precise study of this phenomenon and even explain it, since there seem to be parallels between the immunological data and eye morbidity in onchocerciasis (D'HAUSSY et al., 1972).

4. Conclusions

To make an exhaustive review of all studies of filariasis immunology is a virtually impossible task. We have therefore attempted to pick out the essentials from the main studies, which at the same time as providing many positive results reveal almost as many new unknown features. All this clearly reflects not only the complexity but also the importance of the phenomena concerned. Their applications are both theoretical and practical, particularly in diagnosis and epidemiology. The sero-immunological tests could render valuable services at a time when it is possible to hope for the eradication of various filariases from some parts of the world but when ecological changes or population movements are causing the areas where filariasis is endemic to expand. To be effective in this way, moreover, these tests will need to be further improved and above all better standardized.

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Zusammenfassung

Die Filariosen bilden wahrscheinlich eines der komplexesten Gebiete der serologischen Diagnostik parasitärer Erkrankungen. In diesem Übersichtsartikel ist versucht worden, eine Bilanz der Resultate der letzten 10 Jahre zu ziehen. Im ersten Teil werden die hauptsächlichsten verwendeten Methoden analysiert: Intradermaltest, Komplementbindungsreaktion, passive Haemagglutination, zweidimensionale Geldiffusion, Immunoelektrophorese und Immunofluoreszenz. Vor- und Nachteile der einzelnen Methoden sind dargestellt. Die wichtigsten Probleme der serologischen Filarien-Diagnostik sind des weiteren umfassend diskutiert worden. Diese betreffen vor allem den Wert der verschiedenen Antigene: Spezifische Antigene (z. B. *Wuchereria bancrofti, Onchocerca volvulus)* und Gruppenantigene *(Dirofilaria immitis* und *Dipetalonema vitae)* sowie die Möglichkeiten, diese Antigene zu erhalten. Im gesamten gesehen nehmen die verschiedenen empfindlichen immundiagnostischen Methoden einen großen Platz in der Filariendiagnostik, in posttherapeutischen Kontrollen und in epidemiologischen Untersuchungen ein. Zwar sind die Schlüsse von verschiedenen Autoren, die die gleiche Methode verwenden, manchmal widersprüchlich. Überdies machen häufig Kreuzreaktionen mit verschiedenen Nematoden eine Interpretation der Resultate schwierig.

Résumé

Les filarioses constituent probablement l'un des domaines les plus complexes de la séro-immunologie parasitaire. Dans cette revue générale, on a tenté de faire le bilan des résultats acquis au cours de ces dix dernières années. Dans une première partie, sont envisagées de façon analytique les principales méthodes utilisées: intra-dermo-réactions, tests de fixation du complément, d'hémagglutination passive, de double diffusion en gélose et d'immuno-électrophorèse, d'immunofluorescence. Les avantages et les inconvénients inhérants à chacune de ces méthodes sont successivements considérés. Les principaux problèmes de la séro-immunologie des filarioses sont ensuite discutés de façon synthétique. Ils concernent notamment la valeur des différents antigènes utilisés: antigènes spécifiques *(Wuchereria bancrofti, Onchocerca volvulus* par exemple) ou antigènes de groupe *(Dirofilaria immitis* ou *Dipetalonema vitae* généralement) et leur facilité d'obtention. Dans l'ensemble, les diverses méthodes séro-immunologiques paraissent susceptibles de prendre une large place dans le diagnostic, le contrôle post-thérapeutique, l'étude épidémiologique ou pathogénique des filarioses. Cependant, les conclusions de différents auteurs utilisant pourtant un même test sont parfois contradictoires. En outre, l'interprétation de ces résultats est toujours rendue difficile par l'existence de nombreuses réactions croisées entre les divers nématodes.