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The major radioiodinated cuticular antigens of *Onchocerca gibsoni* microfilariae are neither species nor onchocerca specific

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Summary

The possible role of microfilarial surface (cuticular) antigens in immunodiagnosis of human filarial infections has been assessed using microfilariae (mf) of the cattle parasite *Onchocerca gibsoni.* A Triton X-100 extract of ^{125}I-labeled *O. gibsoni* mf was reacted with a panel of sera from humans infected with *Onchocerca volvulus, Wuchereria bancrofti* and *Schistosoma japonicum* as well as sera from uninfected controls. Results of these immunoprecipitations indicated that sera from humans infected with *O. volvulus* or *W. bancrofti* contain antibody specificities recognising certain of the radioiodinated cuticular proteins of *O. gibsoni* mf. Two-dimensional gel analysis and subsequent autoradiography of these immunoprecipitates showed that 8 radioiodinated proteins recognised by sera from calves injected with *O. gibsoni* mf were also immunoprecipitated by sera from humans infected with either *O. volvulus* or *W. bancrofti.* Thus there appear to be no major radioiodinated cuticular antigens of *O. gibsoni* mf which are species or onchocerca specific.

Key words: Onchocerca gibsoni; microfilariae; species specific cuticular antigens; immunodiagnosis; immunoprecipitation.

Introduction

Immunological tests for diagnosing filarial infections suffer from a lack of specificity due to cross-reactions between filarial spp. to which man is exposed

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in endemic areas (Ambroise-Thomas, 1975; Capron et al., 1968; Fujita et al., 1970; Gidel et al., 1969) and cross-reactions between filariae and other nematodes (Kagan and Norman, 1974). Thus there are high percentages of false positives (up to 30%) associated with the variety of immunological tests available (Ambroise-Thomas, 1980). Moreover, because of the common antigenic features of many filarial species, only a general diagnosis of filariasis rather than identification of a causative species can be obtained by such tests.

Because of the difficulty in obtaining human parasite material, antigen preparations used in existing immunodiagnostic tests for human filarial infections are (with the exception of *Onchocerca volvulus* and *Brugia malayi)* generally derived from filariae parasitic to animals such as *Dirofilaria immitis, Dipetalonema viteae, Breinlia booliati, Setaria digitata* and *Brugia pahangi.* These antigen preparations are complex mixtures of molecules derived from extracts of either adult worms (and which may be partially purified, Sawada et al., 1968; Marcoulis and Grasbeck, 1976; Dissanayake and Ismail, 1980) or from extracts of mf (Lucasse and Hoeppli, 1963; Higashi et al., 1968). Alternatively, fragments or sections of adult worms or intact mf are used as antigen sources for use in indirect immunofluorescence tests (reviewed by Ambroise-Thomas, 1980). Excretory/secretory/metabolic (ESM) antigens, which have proved to be highly specific for immunodiagnosis of *Toxocara canis* infections when harvested from cultured larvae (de Savigny and Tizard, 1977), were not found to be parasite specific when collected from *O. volvulus* mf since cross-reactions with *Loa loa* were observed (Schiller et al., 1980).

Ideally, an immunodiagnostic test for filarial infections would be stage and species specific and thereby detect current infection with a particular parasite rather than exposure to the infective stages of that or other parasites. In an attempt to characterise a more defined nematode antigen preparation, Parkhouse et al. (1981) examined the specificity of two major surface antigens of *Trichinella spiralis* third stage larvae (L3), which had been shown previously to be stage specific antigens (Philipp et al., 1980). Their study showed that a sodium deoxycholate detergent extract of ^{125}I-labeled *T. spiralis* L3, containing the major surface antigens, did not react with a variety of sera from animals chronically infected with other nematode species. The conclusion that was drawn by Parkhouse et al. (1981) from this apparent lack of cross-reactivity was that homologous surface antigens might be suitable for diagnosis of nematode infections.

Further evidence for the stage and species specificity of cuticular antigens of L3 was obtained by Nelson et al. (1971). They showed that anticuticular antibodies raised in rabbits immunised with L3 of *Breinlia sergenti* did not react with mf of the same species nor with L3 of *B. pahangi* or *B. malayi.* The cuticle of L3 of *D. viteae* has also been shown to be antigenically different to that of mf of the same species (Weiss and Tanner, 1981). In this paper we report results of experiments designed to examine the question of species specificity of microfi-

larial cuticular (surface) antigens and to assess the potential of cuticular antigens for immunodiagnosis of filarial infections.

Materials and methods

Parasites. Uterine mf were isolated from fragments of *O. gibsoni* adult worms which were dissected from nodules taken from the brisket region of infected cattle. The isolated mf were purified using a Ficoll-paque method (Forsyth et al., 1981a).

Antisera. Sera were obtained from 2 calves given multiple injections of uterine mf subcutaneously and shown to have no detectable skin mf. Sera from *O. volvulus*-infected humans from Mexico were provided by Dr. Mario Philipp (National Institute for Medical Research, Mill Hill, London, U. K.). Sera from humans living in a region of the Philippines endemic for *W. bancrofti* were provided by Dr. David I. Grove (Department of Medicine, University of Western Australia, Nedlands, W. A.). Another group of sera from humans infected with *W. bancrofti* was collected by Dr. R. Coppel (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) in Madang, Papua New Guinea (PNG). Sera from *S. japonicum*-infected Philippine patients and uninfected controls were provided by Dr. E. G. Garcia (Institute of Public Health, Manila, Philippines). Serum from a Melbourne child with suspected toxocariasis was also used in this study.

Radioiodination of microfilariae. 10^5 uterine mf were ^{125}I-labeled and subsequently solubilized in 1.5% Triton X-100 as described in Forsyth et al. (1981b).

Immunoprecipitation of Triton X-100 extract of ^{125}I-labeled microfilariae. Solubilized ^{125}I-labeled mf (2×10^5 trichloracetic acid (20%) precipitable counts per minute of the supernatant of Triton X-100 extracts) was 'precleared' with 100 μl of a 10% (v/v) of heat killed and formalin fixed *Staphylococcus aureus* of the Cowan I strain (Commonwealth Serum Laboratories, Parkville, Victoria, Australia). The cleared extract was then added to 10 μl of the appropriate bovine or human serum and incubated for 2 h on ice. Immune complexes were isolated by binding to protein A bearing *S. aureus* as described by Kessler (1975). Precipitated immune complexes were washed 3 $\times$ in a 0.05 M Tris-HCL buffer, pH 8, containing 0.05% Triton X-100, 0.15 M NaCl, 0.05 M EDTA as described by Kessler (1975) with a tube change before the last wash, and counted using an autogamma counter. Immunoprecipitates were then analysed by two-dimensional gel analysis according to the method of O'Farrell et al. (1977) using the solubilization and autoradiographic procedures outlined in Forsyth et al. (1981b). Exposure times for gels were either 7 or 16 d.

Results

Sera from a cow infected with *O. gibsoni* and a calf injected with *O. gibsoni* mf immunoprecipitated $>10\%$ of the radioactivity (counts per minute, cpm) in Triton X-100 extracts of ^{125}I-labeled *O. gibsoni* mf using the *S. aureus* method. Moreover, radioiodinated proteins were readily detected using two-dimensional gels to analyse these immunoprecipitates. In contrast, $<5\%$ of cpm were immunoprecipitated by immunoglobulins from a calf not exposed to *O. gibsoni* (Table 1). Relatively high amounts of radioactivity were also immunoprecipitated by sera from 5 *O. volvulus*-infected patients, the mean immunoprecipitated cpm of this group (23.1 ± 3.4) exceeding that of sera from uninfected humans in Melbourne and the Philippines (5.7 ± 0.5). Sera from 3 patients known to be infected with *S. japonicum* immunoprecipitated $<10\%$ of cpm, and, as with sera from uninfected individuals, no radioiodinated proteins could be detected by two-dimensional gel analysis and autoradiography (after 16 days exposure of

Table 1. Radioactivity immunoprecipitated by various human and bovine sera reacted with Triton X-100 extracts of ^{125}I-labeled microfilariae of *Onchocerca gibsoni*

Serum donor	Percent cpm immuno-precipitated	Detection of labeled proteins by two-dimensional gel analysis
O. gibsoni-infected cow	10.7	+*
O. gibsoni mf-injected calf	22.0	+
Uninfected calf**	3.2	–*
O. volvulus-infected humans (5 sera)	15.4, 17.0, 20.3, 30.6, 32.2	+
Suspected toxocariasis child	8.4	+
S. japonicum-infected humans (3 sera)	8.1, 8.6, 9.6	–*
Uninfected humans (Melbourne and Philippines, 6 sera)	4.8, 5.0, 5.2, 5.4, 5.5, 8.1	–*

* Exposure times of autoradiographs = 16 days, all others 7 days

** Purified IgG (Forsyth et al., 1981b) at an amount equivalent to the level of IgG in infected cattle sera was used in this control immunoprecipitation

the gels). However, the serum from a child with suspected toxocariasis immunoprecipitated approximately 8% of cpm and proteins were detected on gels (see below).

Results presented in Table 2 demonstrate that sera from 10 apparently uninfected individuals living in an area of the Philippines which is endemic for *W. bancrofti*, immunoprecipitated from 2.2 to 15.4% of the radioactivity in Triton X-100 extracts of ^{125}I-labeled *O. gibsoni* mf. Seven of these 10 sera (those with the highest percentages of immunoprecipitated cpm) yielded precipitates in which labeled proteins were detected by two-dimensional gel analysis. Labeled proteins on gels were also detected using sera from patients in the Philippines or PNG with elephantiasis or microfilaremia, for immunoprecipitation of *O. gibsoni* mf proteins, but not using sera from 2 patients with elephantiasis *plus* microfilaremia.

Two-dimensional gel analysis of the spectrum of radioiodinated proteins recognised by sera from *O. volvulus*-infected patients (Fig. 1 C) reveals that the pattern of immunoprecipitated proteins was virtually identical to that observed for sera from 2 calves injected with *O. gibsoni* mf (Fig. 1 B). From the total of approximately 25 major radioiodinated proteins solubilized in the Triton X-100 extract of ^{125}I-labeled mf (Fig. 1 A), 8 were shown to be immunoprecipitated by both the 2 above mentioned types of sera.

Sera from *W. bancrofti* infected patients with elephantiasis and 7 out of 10 patients living in a *W. bancrofti* endemic area who had no clinical signs or symp-

Table 2. Radioactivity immunoprecipitated by human filariasis sera reacted with Triton X-100 extracts of ^{125}I-labeled microfilariae of *Onchocerca gibsoni*

Origin of sera	Clinical status of patients	Microfilariae counts per ml of blood	Percent cpm immunoprecipitated	Detection of labeled proteins by two-dimensional gel analysis
Philippines (10 sera)	No signs of filariasis	0	2.2, 4.3, 5.2	–
			6.9, 8.5, 8.6, 9.8,	
			10.3, 12.3, 15.4	+
Philippines (6 sera)	Elephantiasis	0	6.0, 9.1, 9.5,	+
			9.5, 12.2, 21.8	
Philippines (2 sera)	Elephantiasis plus	1200	8.5	–*
	microfilaremia	330	5.0	–*
Philippines (4 sera)	Microfilaremia	150	4.4	+*
		300	7.2	+*
		150	7.5	+*
		1680	8.5	+*
PNG (3 sera)	Elephantiasis	0	10.2, 10.4, 21.8	+
PNG (5 sera)	Microfilaremia	100	5.2	+*
		6 040	9.9	+*
		10 800	11.7	+*
		100	13.4	+*
		600	15.2	+*

* Exposure times of autoradiographs = 16 days, all others 7 days

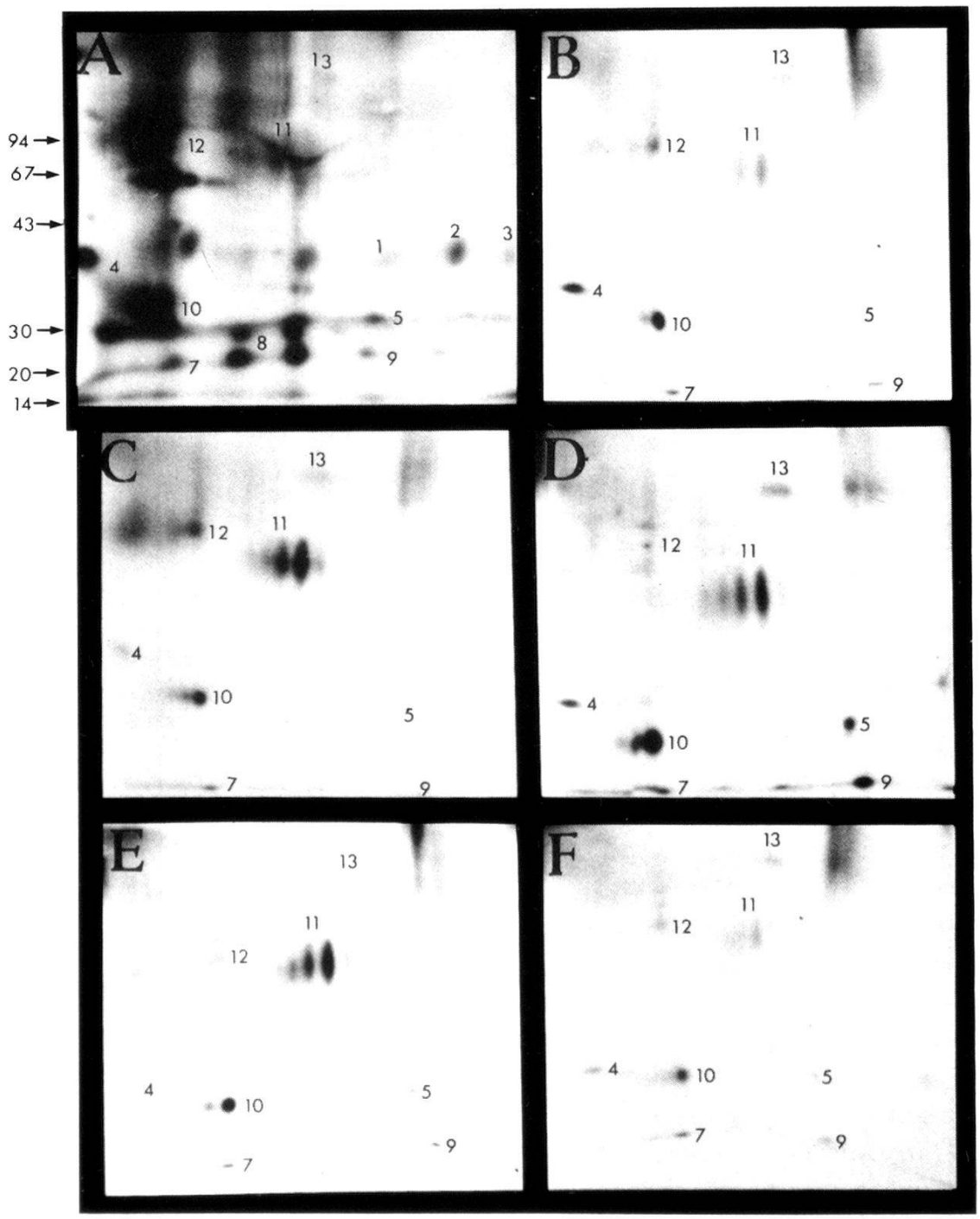

Fig. 1. Autoradiographs of two-dimensional gels (under reducing conditions) of the Triton X-100 extract of ^{125}I-labeled mf (A) and immunoprecipitates of that extract (B–F). A representative immunoprecipitation pattern with a serum from each of the following groups of calves or humans is shown: 2 calves injected with *O. gibsoni* mf (B), 5 humans infected with *O. volvulus* (C), 25 humans living in areas endemic for *W. bancrofti*, 9 of whom had elephantiasis without microfilaremia (D), 7 of whom had no clinical signs or symptoms of filariasis (E), and 9 of whom were microfilaremic (F). Numbers in Fig. 1 A represent molecular weight standards: phosphorylase B (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000) soybean trypsin inhibitor (20,000) and lactalbumin (14,4000).

toms of filariasis, recognised 8 of the same radioiodinated proteins as did homologous calf serum (Figs. 1 D, E). The relative intensity of the immunoprecipitated radioiodinated proteins appeared to be the same for the 4 above mentioned groups of sera (Figs, 1 B, C, D, E), i.e. the major radioiodinated protein antigens in all 4 cases were proteins designated 5, 7, 9, 10 and 11 of apparent Mr 30,000, 22,000, 22,000, 32,000 and 60,000, respectively. The 4 clustered proteins designated 8 in Fig. 1 A were only weakly immunoprecipitated by these 4 groups of sera and are poorly visualized in Figs. 1 B, C, D, E.

The percentage of cpm immunoprecipitated by sera from Philippine patients infected with *W. bancrofti* who were microfilaremic was generally lower than for sera from elephantiasis patients who were amicrofilaremic. This difference was reflected in relative exposure times for the autoradiographs in Figs. 1 D and 1 F, i.e. 7 d compared to 16 d. Sera from microfilaremic Philippine patients recognised the same spectrum of radioiodinated proteins (Fig. 1 F) as did other microfilaremic individuals from PNG. Again, they were the same 8 radioiodinated proteins recognised by the homologous *O. gibsoni* sera (Fig. 1 B). However, the relative intensity of immunoprecipitated proteins on autoradiographs was somewhat different to that found for proteins immunoprecipitated by the homologous serum as well as sera from either *O. volvulus* infected humans or from *W. bancrofti* infected patients with elephantiasis. Proteins designated 5, 7, 9, 10, and 11 in Figs. 1 B, C, D, E were the major antigens recognised. However, the protein complex designated 11 in Fig. 1 F was immunoprecipitated to a lesser extent by serum from the microfilaremic individuals. The serum from a child with suspected toxocariasis immunoprecipitated radioiodinated proteins 10 and 13 in Fig. 1 A. This result with a single serum suggests that these two antigens may be shared between widely disparate systemic nematodes.

Discussion

Immunodiagnostic tests for human filarial infection based on the detection of antibodies would ideally utilize antigens specific for adults or microfilariae (i.e. established stages of the parasite life cycle). Conversely, immunodiagnostic tests for detection of exposure to filarial parasites (and perhaps resistance to establishment) would ideally utilize antigens specific for the infective (L3) stage. These considerations also apply to other important human parasitic infections such as schistosomiasis. Both types of test would have considerable use in epidemiological studies on filariasis (including onchocerciasis) and the monitoring of control programmes. Naturally, detection of circulating or urinary antigens would be more useful than detection of antibodies in identification of currently infected individuals.

The immunoprecipitation results from Tables 1 and 2 demonstrate that sera from humans infected with either *W. bancrofti* or *O. volvulus* contain anti-

bodies directed against radioiodinated cuticular antigens of *O. gibsoni* mf. These positive reactions are unlikely to result from the presence of antibodies in human sera directed to bovine material because ^{125}I-labeled *uterine* mf do not appear to have detectable amounts of bovine serum albumin or bovine immunoglobulins adsorbed to their surface (Forsyth et al., 1981b). Moreover, sera from patients infected with *S. japonicum*, or not known to be exposed to any filarial parasites, immunoprecipitated low amounts of radioactivity and labeled proteins were lacking in two-dimensional gel analyses of such immunoprecipitates (Table 1).

Sera from 10 individuals living in an area endemic for *W. bancrofti* but having no clinical signs or symptoms of filariasis, showed variability in anti *O. gibsoni* mf reactivity; 7 were clearly positive, 3 negative. Four possibilities can be advanced to account for the reactions seen with the 7/10 sera: (1) patients have recovered from infection due to development of anti mf immunity detected by the presence of cross-reacting antibodies to labeled *O. gibsoni* mf antigens in sera. It is well known that the presence of anticuticular or antisheath antibodies in various filarial infections is associated with amicrofilaremia (McGreevy et al., 1980; Piessens et al., 1980), (2) the antigens of *O. gibsoni* are not stage (or onchocerca) specific and are immunoprecipitated by antibodies induced as a result of exposure to *W. bancrofti* L3, (3) parasitological examination of these patients gave false negative results, and (4) patients were infected with another cross-reacting parasite (e.g. an intestinal nematode). Such cross-reactions cannot be extensive since the schistosomiasis japonica sera used in the above experiments were from patients who were also infected with ascaris, trichuris and/or hookworm. Similar parasitological information on the donors of the 10 sera from the *W. bancrofti* endemic area is not available.

The 8 major radioiodinated cuticular protein antigens which were precipitated by sera from calves injected with *O. gibsoni* mf were also precipitated by sera from *O. volvulus* and *W. bancrofti* infected humans. Thus, these major antigens are neither species nor onchocerca specific. The results of this study strongly suggest that microfilarial cuticular antigens will be of no use in parasite-specific (cf. filaria-specific) immunodiagnosis in human filariasis. Supporting this conclusion is the work of Dissanayake and Ismail (1980), who have shown that 2 antigen fractions extracted from *S. digitata* adult worms cross-reacted with surface antigens of *W. bancrofti* microfilariae.

As outlined in the Introduction, the studies of Parkhouse et al. (1981) on the specificity of the major surface proteins are encouraging in terms of development of specific immunodiagnostic tests for exposure. The studies of de Savigny and Tizard (1979) also indicate that the excretory/secretory/metabolic (ESM) products of L3 of *Toxocara canis* have high specificity. These results have been confirmed using *T. canis*, *Toxascaris leonina* and *Ascaris suum* in a mouse system (Nicholas W. L., Mitchell G. F. and Stewart A., unpublished observations).

With respect to the use of *O. gibsoni* mf cuticular antigens as heterologous antigens for serological diagnosis of filarial infections, at least some appear to be filaria specific. If it can be shown that certain of the 8 radioiodinated cuticular antigens are *stage* specific for mf of various filariae (and perhaps do not cross react with antigens responsible for induction of host protective responses), then they may be suitable for detection of current or recent infection. Finally, the indirect serological evidence for extensive sharing of antigens between filarial parasites suggests that any cross reactive host protective responses which are eventually identified, may well be induced by at least some of the heterologous antigens after isolation (see Nelson, 1974 for discussion on zooprophylaxis).

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