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**Different suitability of 3 filarial antigens
(*Litomosoides carinii*, *Dipetalonema viteae*, *Dirofilaria immitis*)
to act as allergens in the Passive Cutaneous Anaphylaxis Test
and to serve as antigens in an ELISA in the course
of experimental filarial infections (*L. carinii*, *D. viteae*,
Brugia malayi, *B. pahangi*) of *Mastomys natalensis***

H. ZAHNER, G. REINER

Summary

Crude extracts of adult worms of 3 different filariae (*Litomosoides carinii*, *Dipetalonema viteae*, *Dirofilaria immitis*) were evaluated for their suitability to serve as allergens in the Passive Cutaneous Anaphylaxis Test (PCA) and as antigens in an ELISA which detected mainly IgG antibodies. Studies were done in the course of 4 different filarial infections (*L. carinii*, *D. viteae*, *Brugia malayi*, *B. pahangi*) of *Mastomys natalensis*, using sera from different times after infection up to 350 days p.i. – In the PCA *L. carinii* and *D. viteae* antigens, apart from *L. carinii* infection caused reactions to a similar degree. In the *L. carinii* infection the homologous antigen was more effective. The *D. immitis* antigen was clearly less effective: high titres were found only during the early prepatency of *D. viteae* and *Brugia* infections and during the early patency of *L. carinii* and *D. viteae* infections. In all other cases, if at all, it led to weak reactions only. – In the ELISA different time courses were obtained as well. In *Brugia* infections values obtained with *D. viteae* and *D. immitis* antigens were significantly correlated but were not related to those obtained by the *L. carinii* antigen. However, the *L. carinii* antigen detected high levels of antibodies especially during prepatency of *B. malayi*, *B. pahangi* and *D. viteae* infections. No relations were found between the antigens for the *D. viteae* infection. In the case of the *L. carinii* infection the values of all 3 antigens were significantly correlated.

Key words: experimental rodent filariasis; filarial antigens; *Mastomys natalensis*; PCA; ELISA.

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Introduction

The availability of homologous antigens for immunological studies in filarial infections is not guaranteed in each case since the isolation of sufficient amounts of parasites may be difficult. As an alternative – especially in human disease (see Kagan, 1963; Ambroise-Thomas, 1974) but also in experimental infections (e.g. Benjamin and Soulsby, 1976; Gusmao et al., 1981; Tandon et al., 1983) often antigens are used from filariae which are more easily available as for example from *Litomosoides carinii*, *Dipetalonema viteae* and *Dirofilaria immitis*, relying on sufficient antigenic cross reactivities between the species.

However, recent studies on homocytotropic antibodies in the course of various experimental filarial infections of *Mastomys*, using *L. carinii* antigen pointed at marked differences in the allergic capacity, in particular between this antigen and *D. immitis* antigen (see Benjamin and Soulsby, 1976; Gusmao et al., 1981; Zahner et al., 1983).

The present studies therefore were carried out to evaluate differences in the sensitivity of antigens derived from 3 filarial species, *L. carinii*, *D. viteae*, *D. immitis*, to act as allergens in various phases of experimental filarial infections. These data were compared with data obtained by a mainly IgG detecting ELISA. As experimental system *L. carinii*, *D. viteae*, *Brugia malayi* and *B. pahangi* infections of *Mastomys natalensis* were used.

Material and Methods

Animals: *Mastomys natalensis* (strain GRA Giessen) were conventionally bred at the institute and kept and fed as described earlier (Schuster et al., 1973).

Infections: Animals were infected with *Litomosoides carinii* by allowing infected mites, *Bdellonyssus bacoti*, to suckle (Lämmler et al., 1968). For infections with *Dipetalonema viteae* 3rd stage larvae were isolated from *Ornithodoros moubata* 30 days after the ticks had been fed on infected jirds (Sänger and Lämmler, 1979). 70 larvae were injected subcutaneously in the neck region. Infective stages of *Brugia malayi* were isolated from *Aedes aegypti* 11 days after an infective blood meal. Larvae from *B. pahangi* were collected from *A. togoi*. For *Brugia* infections 80 larvae were injected subcutaneously in the neck region (Sänger et al., 1981).

Blood for serum collection was taken from the retroorbital venous plexus of the animals at designated times after infection. Different groups were bled at the different dates. Sera were isolated after clotting (1 h, room temperature) and centrifugation ($15,000 \times g$, 4 min) and stored at -40°C until use. For the investigation pools were made from 8–12 individual sera.

Antigens

L. carinii adult worm antigen: Adult worms were isolated from infected *M. natalensis*, washed intensively and stored lyophilized. For antigen preparation worms were ground in a mortar, suspended in a small amount of distilled water and homogenized (20,000 revs/min, 2 min, 4°C , N_2 -atmosphere). The material was extracted at a final concentration of 1:100 w/v in distilled water (18 h, 4°C). After centrifugation ($25,000 \times g$, 30 min, 4°C) the supernatant was used as antigen.

D. viteae adult worm antigen: Adult worms were isolated from infected *Mastomys* and processed as *L. carinii* but using saline instead of distilled water.

D. immitis adult worm antigen: Adult worms were isolated from infected dogs¹. The antigen was prepared analogously to the *D. viteae* antigen.

Passive Cutaneous Anaphylaxis Test (PCA) used normal *Mastomys* as recipients. 0.05 ml of undiluted serum and twofold serial dilutions beginning at 1:5 were injected intradermally in anaesthetized (50 mg pentobarbital sodium/kg i.p.) animals. The animals were challenged 72 h later by intravenous injection of either *L. carinii*, *D. viteae* or *D. immitis* antigens diluted in saline containing 0.6% Evans Blue. In all cases 1 mg antigen (based on the dry weight after lyophilization) was injected in 0.4 ml/100 g bodyweight.

Recipients were killed with chloroform after 30 min. The reaction was read from the internal side of the skin. Reactions of at least 4 mm in diameter were regarded as + reaction indicating the endpoint of the titration (skin reactions occurred occasionally after the injection of normal serum. They never exceed 2–3 mm in diameter).

All tests were carried out in duplicate.

Enzyme Linked Immuno Sorbent Assay (ELISA): Polystyrene test tubes (Greiner, Nürtingen) were sensitized overnight, at room temperature, with 0.2 ml antigen solution/well (20 µg protein/ml of a 0.05 M NaCO₃ buffer, pH 9.6, containing 0.02% NaN₃). The tubes were washed three times with PBS containing 0.05% Tween 20 (Serva, Heidelberg). 0.2 ml test serum, diluted 1:400 in PBS (containing 0.05% Tween 20 and 0.02% NaN₃) were allowed to react for 2 h at room temperature. After 3 washings 0.2 ml conjugate (anti-Mn-Ig-IgG-POD) were added and allowed to react for 2 h at room temperature. After 3 further washings 0.2 ml substrate buffer were added (0.4% o-phenylenediamine, 0.012% H₂O₂ in phosphate-citrate buffer (25.7 ml 0.2 M dibasic sodium phosphate, 24.3 ml 0.1 M citric acid, 50 ml H₂O), pH 5.0). The reaction was stopped after 30 min by 1 ml 0.5 N H₂SO₄. Extinctions were read at 498 nm.

Tests were performed in duplicate.

The conjugate anti-Mn-Ig-IgG-POD against immunoglobulins from *Mastomys* (Mn-Ig) was prepared by isolating immunoglobulins from the serum by two times repeated precipitation in 42.5% (NH₄)₂SO₄. Antiserum was prepared in goats. The IgG fraction of the goat antiserum was obtained by precipitations as mentioned above and a chromatography of the redissolved (PBS) precipitate on a Sephadex G-200 (Pharmacia, Freiburg) column. The second peak was pooled and regarded as partially purified IgG. According to the method of Wilson and Nakane (1978) aliquots of this fraction were conjugated to horseradish peroxidase (POD). For the test the conjugate was diluted 1:2000 in PBS containing 0.05% Tween 20.

Statistical analyses: The significance of correlations was tested after calculating the correlation coefficient (*r*) (Sachs, 1978).

Results

Passive Cutaneous Anaphylaxis Test (PCA) (Fig. 1)

L. carinii infection: Using *L. carinii* antigen more or less constant titres around 1:10 were found throughout the observation period. *D. viteae* antigen induced PCA reactions of lower titres during prepatency. It was ineffective in the case of sera dating from the early patency and from the 220th day p.i. but evoked weak reactions with sera which were isolated 90, 140 and 350 days p.i. *D. immitis* antigen induced reactions with neat sera dating from the end of the prepatency and the early patency. A PCA titre of 1:5 was observed with serum dating from the 90th day p.i. In all other cases attempts to induce skin reactions with *D. immitis* antigen failed.

¹ Part of the material (lyophilized worms) was kindly supplied by Dr. D. Weiner, Philadelphia, and Dr. J. McCall, Atlanta, USA.

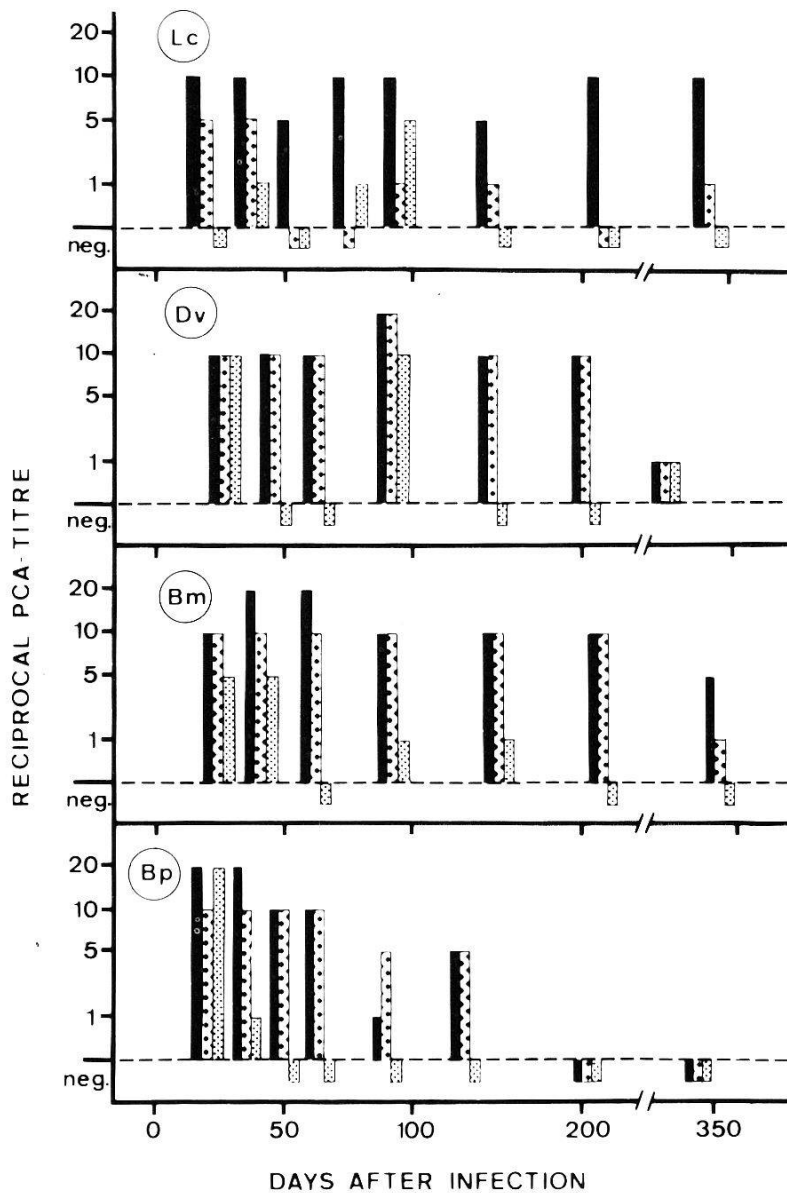


Fig. 1. Reaginic antibody titres (Passive Cutaneous Anaphylaxis Test: PCA) of sera of *Litomosoides carinii* (Lc), *Dipetalonema viteae* (Dv), *Brugia malayi* (Bm) and *B. pahangi* (Bp) infected *Mastomys natalensis* at various times after the infection, using *L. carinii* antigen ■, *D. viteae* antigen ▤ and *Dirofilaria immitis* antigen ▥.

D. viteae infection: Both the homologous antigen and the *L. carinii* antigen led to identical PCA titres. The highest titres (1:20) occurred in the early patency. Titres were found reduced at the last bleeding date 260 days p.i. Using *D. immitis* antigen antibodies could be demonstrated only in the early prepatency, 90 days p.i., i.e., when the other antigens led to maximum titres, and at the end of the observation period.

B. malayi infection: Titres between 1:10 and 1:20 were obtained between 15 and 200 days p.i. using *L. carinii* and *D. viteae* antigens, the *L. carinii* antigen leading sometimes to higher titres. About 300 days p.i. the titres were found slightly decreased. *D. immitis* antigen was effective only in the early prepatency,

Table 1. PCA titres of sera of *Brugia pahangi* infected *Mastomys natalensis* observed using *Litomosoides carinii* and *Dirofilaria immitis* antigens

	Reciprocal PCA titres of sera* obtained days after <i>B. pahangi</i> infection							
	14	29	43	57	71	82	106	117
<i>L. carinii</i> Ag	20	40	5	5	5	10	5	5
<i>D. immitis</i> Ag	10	5	0	0	0	0	0	0

* Sera consisted of a pool of 10–12 individual sera

towards the beginning and in the early patency, i.e. 15, 36, 90 and 130 days, respectively p.i. but it induced only weak reactions.

B. pahangi infection: Using *L. carinii* and *D. viteae* antigens similar antibody levels and time courses were detected. Maximum titres occurred during the early prepatency; titres were reduced after the beginning of patency. Sera which were isolated 200 and 300 days p.i. did not contain detectable, reaginic antibodies. Using *D. immitis* antigen reagins could be demonstrated on two occasions, i.e. 20 days p.i. and, already reduced, 40 days p.i. By a second experiment with different sera, isolated in the course of the prepatency and the early patency, *L. carinii* and *D. immitis* antigens were compared and it led to similar data (Table 1).

ELISA (Fig. 2)

A pool of normal sera and a pool of sera from *L. carinii* infected *Mastomys* were used in each test as a negative and a positive reference, respectively. Optical densities at 498 nm of the negative standard were 0.26 (*L. carinii* antigen), 0.32 (*D. viteae* antigen) and 0.30 (*D. immitis* antigen). In the case of the positive standard mean values were 1.70, 1.38 and 1.10, respectively (not included in Fig. 2).

L. carinii infection: Using the homologous antigen during prepatency and early patency continuously increasing antibody levels were observed. Thereafter levels increased more rapidly and led to a maximum on day 210 p.i. In the case of *D. viteae* and *D. immitis* antigens, after a first increase, the antibody content of the sera levelled off during the late prepatency followed by a further increase after the beginning of patency. Similar to antibodies detectable by *L. carinii* antigen a marked rise was observed later than 70 days p.i. but extinction values remained clearly lower than in the case of the homologous antigen – especially when *D. immitis* antigen was used.

Significant correlations between the data obtained for the 3 antigens correspond to similar time courses (Table 2).

D. viteae infection: Relative high levels of antibodies were found already 18 days p.i. Using the homologous antigen at this time slightly lower values were

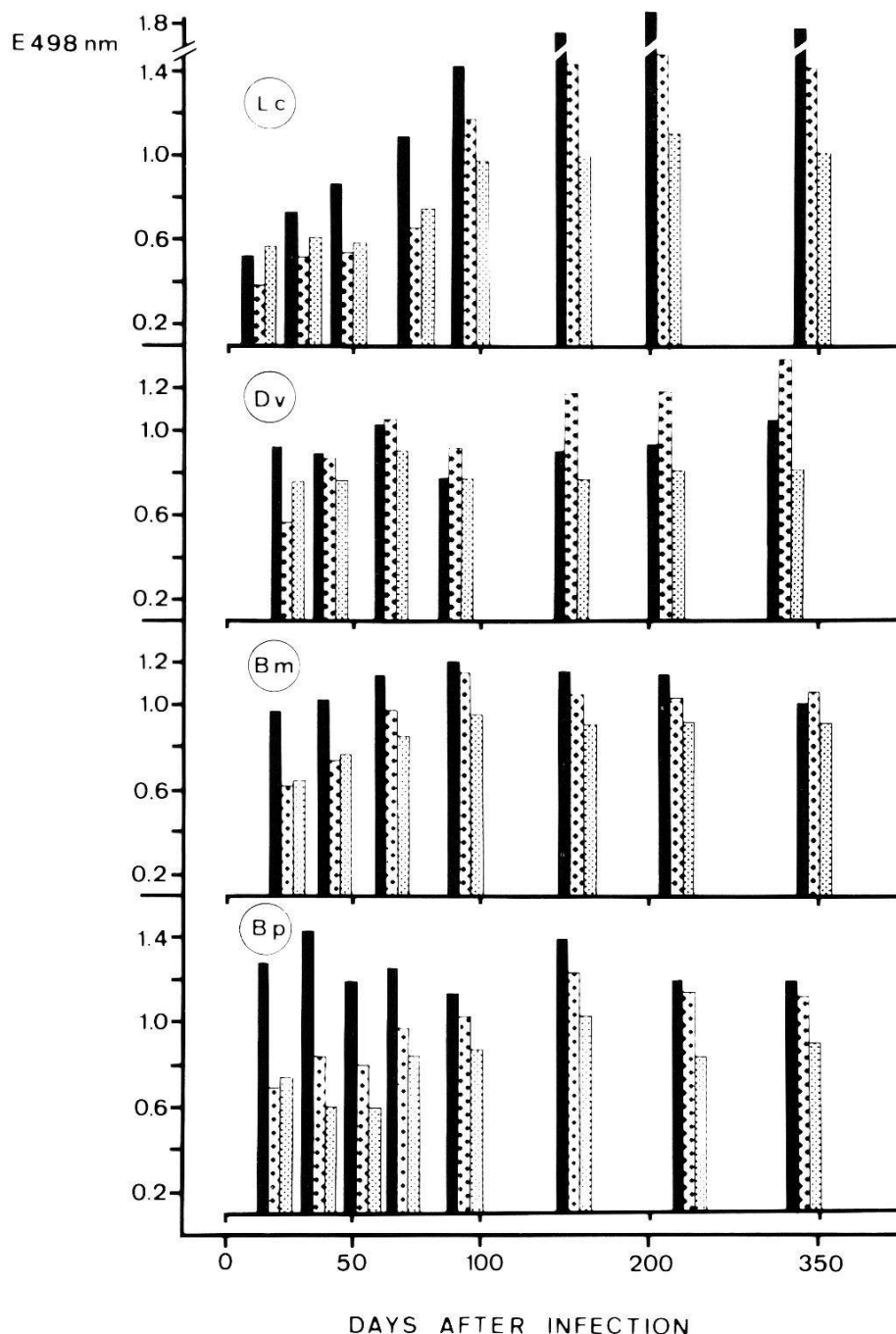


Fig. 2. Antibody levels detected by ELISA in sera of *Litomosoides carinii* (Lc), *Dipetalonema viteae* (Dv), *Brugia malayi* (Bm) and *B. pahangi* (Bp) infected *Mastomys natalensis* at various times after the infection, using *L. carinii* antigen ■, *D. viteae* antigen ▨ and *Dirofilaria immitis* antigen ▩.

found than with *D. immitis* and especially with *L. carinii* antigen. After that, 40 and 60 days p.i., i.e. shortly before and after the beginning of patency, similar antibody levels were observed by the 3 antigens, since only minor variations had occurred regarding antibodies detectable by *L. carinii* and *D. immitis* antigens. During patency antibody levels, which could be demonstrated by the homologous antigen, increased whereas more or less constant time courses were found in other cases.

Table 2. Correlations between ELISA values of sera from *Mastomys natalensis*, isolated between 15 and 350 days after infections with *Litomosoides carinii*, *Dipetalonema viteae*, *Brugia malayi* and *B. pahangi*, obtained by *L. carinii* (Lc), *D. viteae* (Dv) and *Dirofilaria immitis* (Di) antigens

Infection	Correlation coefficient (<i>r</i>) and level of significance when comparing antigens					
	Lc/Dv		Lc/Di		Di/Dv	
	<i>r</i>	p	<i>r</i>	p	<i>r</i>	p
<i>L. carinii</i> (n = 8)	0.98	<0.001	0.97	<0.001	0.98	<0.001
<i>D. viteae</i> (n = 7)	0.53	–	0.42	–	0.29	–
<i>B. malayi</i> (n = 7)	0.74	–	0.71	–	0.99	<0.001
<i>B. pahangi</i> (n = 8)	–0.05	–	–0.07	–	0.85	<0.01

Statistical analyses did not indicate any significant correlation between the extinction values obtained in sera from *D. viteae* infected animals with the 3 antigens (Table 2).

B. malayi infection: An early increase in serum antibody content was found using *L. carinii* antigen. The levels slightly increased until day 90 p.i., thereafter they declined continuously to levels which had already been observed 40 days p.i. In contrast, both *D. viteae* and *D. immitis* antigens pointed at a delayed but continuous increase in antibody levels until day 90. Thereafter, apart from a slight decrease in the case of *D. viteae* antigen more or less constant extinction values were observed.

Data obtained by *D. viteae* and *D. immitis* antigens were significantly correlated, but no statistically relevant relations were found between these and those obtained with *L. carinii* antigen (Table 2).

B. pahangi infection: Using *L. carinii* antigen a high antibody content was found in the early prepatency 28 days p.i. After that levels decreased, followed by a second rise during the early patency, i.e. 90 days p.i. Thereafter slightly decreasing extinction values were observed. When *D. viteae* or *D. immitis* antigens were used, the early occurrence of antibodies was less obvious but later than day 40 antibody levels increased strongly, leading to a maximum on day 135. Thereafter a time course similar to that of *L. carinii* was found.

Data of *D. viteae* and *D. immitis* antigens were significantly correlated but did not show any relations to those obtained by *L. carinii* antigen (Table 2).

Discussion

The present study shows that extracts of *L. carinii*, *D. viteae* and *D. immitis* possess different capacities to react with reagins and IgG antibodies in the course of experimental filarial infections.

Apart from patent *L. carinii* infections, *L. carinii* and *D. viteae* antigens showed a very similar suitability to serve as allergen, i.e. to evoke anaphylactic skin reactions. Reaginic antibodies could be detected throughout *D. viteae* infection – in this case the homologous antigen was not superior to the *L. carinii* antigen – during the prepatency and the first 4 months of patency of *B. malayi* infections and during the prepatency and early patency of *B. pahangi* infections. In the case of *L. carinii* infections the *D. viteae* antigen failed to react with reaginic antibodies which can be found by the homologous antigen during the early patency. The homologous antigen was also clearly superior to the *D. viteae* antigen in the later phase of the *L. carinii* infection.

Less efficacy must be supposed for the *D. immitis* antigen. Indeed, apart from *L. carinii* infection it elicited strong reactions when sera were tested which had been isolated during the early prepatency and reacted with antibodies occurring towards the beginning of patency but it was fairly ineffective at the end of the prepatency and at later phases of patency in the various infections.

However, in spite of the limited capacity of *D. immitis* antigen to act as allergen it was not a generally unsuitable antigen to detect antifilarial antibodies. It reacted in all sera from infected animals which were tested by ELISA and led to high extinction values in the course of patent infections, although levels detectable at this time by *L. carinii* and *D. viteae* antigens were usually higher. Apart from the *D. viteae* infection results obtained by the *D. immitis* antigen were very similar to those which were found with *D. viteae* antigen as demonstrated by the significantly correlated extinction values. Concerning *L. carinii* infections ELISA values were correlated even for all antigens, but in the other cases the *L. carinii* antigen apparently differed from *D. viteae* and *D. immitis* antigens since it led to the highest extinction values and was highly sensitive during the prepatency. The latter became clearly obvious in *Brugia* infections and even in sera isolated from *D. viteae* infected *Mastomys* during prepatency, the *L. carinii* antigen showed extinction values higher than the homologous antigen.

The study shows that at least with regard to reaginic antibodies, the question of the source of a filarial antigen can be crucial. *D. immitis* antigen was clearly less efficient in the PCA, although antigen of this origin has often been used in human filarial infections for intradermal tests – using purified fractions as well – with more or less success (see Smith et al., 1971; Ambroise-Thomas, 1974). Since there is increasing further evidence that the allergic cross reactivity of *D. immitis* extracts and several filariae which are pathogenic for man is limited (Grove et al., 1977; Ottesen et al., 1979; Schiller et al., 1980; Weller et al., 1980) the suitability of such antigens should be investigated very critically. It is of interest that more recent studies by Weiss et al. (1981, 1982) suggest that filariae antigens which induce IgE antibodies, i.e. allergens, are generally more species specific than those which induce IgG antibodies.

As far as IgG antibodies are concerned such limitations might be less

important under a practical point of view. Investigations done in human onchocerciasis, analogously to the present results, did not reveal clear differences in the sensitivity of the 3 antigens (Tandon et al., 1983). Other comparative studies carried out in human filariasis by enzyme immunoassays indeed favour homologous antigens or antigens from more closely related species (Bartlett et al., 1975; Speiser and Weiss, 1979; Weiss et al., 1981) but did not report similar differences between antigens as observed in the present study with regard to their allergic capacity. However, further experiments must clarify whether the superior sensitivity of the *L. carinii* antigen in the course of prepatent infections can be used for diagnostic purposes.

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