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Sharing of antigens among filarial species revealed by antibody dependent cell-mediated reactions †

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Abstract. Antisera raised in albino rats against microfilariae of *Litomosoides carinii, Brugia pahangi, Brugia malayi* and sera from *Bancroftian elephantiasis* patients promoted rat neutrophil-mediated adherence and cytotoxicity to the microfilariae. Pre-treatment of the immune sera, with microfilarial antigen at a final concentration of 5 and 25 μg per ml blocked cellular adherence and cytotoxicity to the microfilariae indicating the presence of cross-reactive antibodies. The heterologous immune sera were effective in eliminating the circulating *Litomosoides carinii* microfilariae in *Mastomys natalensis.*

Keywords. Microfilariae; antigen; cross-reaction; antibody-dependent cellular cytotoxicity.

Introduction

The onset of an antibody that promoted cell-mediated killing of microfilariae is associated with the termination or reduction of circulating microfilariae in albino rats infected with *Litomosoides carinii* (Subrahmanyam *et al.,* 1976), in hamsters with *Dipetalonema viteae* (Weiss, 1978), in cats with *Brugia pahangi* (Ponnudurai *et al.,*1974), in rhesus monkeys with *B. malayi* (Aiyar *et al.,* 1982) and in humans with *Wuchereria Bancroftian* (Mehta *et al.,* 1981).

The antibodies seem to be directed to the sheath and/or cuticle of the parasites. There is some evidence to indicate that certain antigenic determinants are shared between different filarial parasites. The present report further substantiates the sharing of antigens between *L. carinii, B. pahangi, B. malayi* and *W. bancrofti* as revealed by the antibody-dependent cell-mediated cross reactions.

Materials and methods

Filarial infections

Rats were infected with *L. carinii* by infective mites, *Bdellonyssus bacoti.* Methods for the maintenance of the infection and monitoring it by means of microfilarial counts in

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Abbreviations used: ADCC, Antibody-dependent cellular cytotoxicity, Mf, microfilariae, AIRS, actively immunised rat serum, fNRS, fresh normal rat serum.

the peripheral blood smears were described by Bagai and Subrahmanyam (1968). *B. pahangi* and *B. malayi* infections in *Mastomys natalensis* ('GRA' strain) were maintained as described by Sanger *et al.* (1981).

Media

RPMI-1640 [RPMI-1640 (GIBCO) was buffered with HEPES (25 mM)], MEM/FCS/DNase (minimum essential medium (MEM) (GIBCO) was supplemented with 10% foetal calf serum (FCS) (GIBCO) inactivated at 56°C for 1 h. DNase (SIGMA) was added to the medium at a concentration of 30mg/L) and Tryode/Gelatin/DNase (tyrode solution was prepared with 0·1 % gelatin and 30 mg/L of DNase) were supplemented with penicillin (100 I.U./ml) and streptomycin (100 μg/ml) and used. Ally media were sterilised by passing through 0·22 μm millipore filter unit (Millipore Corp., Bedford, USA) and stored at 4°C until use.

Microfilariae

Microfilariae (MF) of *L. carinii* (Lc-Mf), *B. pahangi* (Bp-Mf) and *B. malayi* (Bm-Mf) were isolated from the blood of infected *Mastomys natalensis* by density gradient centrifugation over iso-osmotic Percoll® (Pharmacia) as described by Chandrashekar *et al.* (1984).

Microfilarial antigens

The microfilariae were sonicated in PBS at 4°C (20 KHz; 8 ×30 sec), The sonicated preparation was spun at 20,000 rpm for 30 min at 4°C. The supernatants from each fraction was dialysed and the protein content determined (Bradford, 1976) and stored in suitable aliquots at – 70°C.

Sera

Antisera were prepared from the blood of 4–6 weeks old male albino rats ("Wistar" strain) immunised with sonicated Mf (actively immunised rat serum, AIRS). Rats were immunised with three intramuscular injections, 10-15 days apart of microfilarial sonicates ($1{\cdot}8$ –$2{\cdot}5 \times 10^5$ Lc-Mf or $0{\cdot}5$ –$1{\cdot}0 \times 10^5$ Bp-Mf or Bm-Mf), emulsified in Freund's complete adjuvant, and bled 2–3 weeks after the last booster dose. Normal rat serum (fNRS) was freshly prepared before each experiment. The blood drawn by cardiac puncture, was allowed to clot at 37°C for half an hour before the serum was separated by centrifugation at 2000 *g* for 10 min at 4°C. Sera from patients with elephantiasis due to *Wuchereria bancrofti* (ELS) were isolated from venous blood.

Cells

Neutrophils: Peritoneal exudate cells (PEC) were obtained from two months old albino rats, 16–20 h after intraperitoneal injection of 5 ml of 12·5% casein. The peritoneal cavities were washed out with 20 ml of MEM containing 10 units/ml of heparin. The cells were washed twice with MEM/FCS/DNase and finally suspended in 2 ml of the medium and subjected to isopycnic discontinuous gradient centrifugation

(Vadas *et al.,* 1979) comprising 18, 20, 22, 24 and26 % metrizamide concentrations. The 22/24 metrizamide interface yielded 95–100% pure neutrophils.

Eosinophils

PEC obtained from normal rats, 48–72 h after intraperitoneal injection of 5 ml of paraffin oil, were washed with MEM/FCS/DNase and layered over metrizamide gradients. Eosinophils of greater than 95 % purity were obtained at 24/26 metrizamide interface.

The viability of the cells was assessed by trypan blue exclusion technique.

Treatment of immune sera

Lc-Mf AIRS, Bp-Mf AIRS, Bm-Mf AIRS and ELS were treated with microfilarial antigen. For this purpose, 1 ml of each of the immune serum was separately treated with either Lc-Mf antigen, Bp-Mf antigen or Bm-Mf antigen taken in 1 ml of RPMI-1640 medium at a final concentration of 5 and 25 μg of antigen per ml. The mixture was incubated at 4°C overnight and was then spun at 20,000 rpm. The supernatant was used in the ADCC assay. The control sera were similarly diluted with 1 ml RPMI-1640.

Culture conditions and cell-adherence assay

Fiftyμl of RPMI containing Mf (100 of *B. pahangi* or *B. malayi* or200 of *L. carinii*) were incubated with 50 μl of cells (5×10^5) and 50 μl of f NRS and/or AIRS in a final volume of 0·2 ml in a flat bottom 96 well-microtiter plate (Costar, Massachusetts, USA). The plates were incubated in an atmosphere of 5 % CO_2,95 % air for 4–16 h at 37°C. After incubation, the samples were examined microscopically for cellular adherence and cytotoxicity to Mf as described by Mehta *et al.* (1981).

Passive transfer experiment

Three to four weeks old male *Mastomys natalensis* were used in this experiment. Five groups, each comprising 6 animals, were given intraperitoneal injection of $1{\cdot}25 \times 10^6$ Lc-Mf suspended in RPMI-1640. The microfilaremia was monitored (as described by Bagai and Subrahmanyam, 1968) for 3 days. On day 3, 4 groups were treated intraperitoneally with 1·5 ml of Lc-Mf AIRS, Bp-Mf AIRS, Bm-Mf AIRS and ELS respectively. The 5th group was given fNRS that served as the control group. After treatment, the microfilaremia was monitored till day 14 in all groups.

Results

Cell-mediated adherence and cytotoxicity to Mf with different immune sera

L. Carinii Mf: ADCC assay was carried out with *L. carinii* Mf in the presence of fNRS, Lc-Mf AIRS, Bp-Mf AIRS, Bm-Mf AIRS and ELS, using neutrophils and eosinophils as effector cells. The data are presented in table 1. As seen from the table 1, except fNRS, the rest of the sera promoted significant cellular adherence and cytotoxicity to the Mf. However the cytotoxic effect due to ELS was comparatively very low. Eosinophils,

Table 1. Cell mediated adherence and cytotoxicity to microfilariae of *L. carinii* with different rat sera

Cells	%	Sera				
		fNRS	Lc-Mf AIRS	Bp-Mf AIRS	Bm-Mf AIRS	ELS
Neutrophils	Adherence	0	100	100	100	73 ± 3
	Cytotoxicity	0	76 ± 3	71 ± 2	75 ± 10	14 ± 2
Eosinophils	Adherence	0	100	100	100	76 ± 4
	Cytotoxicity	0	7 ± 2	2 ± 1	16 ± 2	7 ± 2

Mean ± SEM of 3 experiments.

although readily adhering to the Mf, did not exert much cytotoxicity to the parasites.

Brugia Mf : The results with Mf from *B. pahangi* and *B. malayi* were similar to those obtained with *L. carinii* (data not shown) except that fNRS alone promoted some adherence of cells without any apparent cytotoxicity to the parasites. Homologous sera as well as Lc-Mf AIRS were equally potent in the ADCC reaction.

ADCC inhibition assay

To ascertain the extent of cross reaction, the immune sera were pretreated with either of the 3 microfilarial antigens, Lc-Mf, Bp-Mfand Bm-Mfat a final concentration of 5 and 25μg of antigen/ml as described under 'methods'. The treated sera were then used in the ADCC reaction against microfilariae of the 3 species using neutrophils as effector cells. The sera were found to markedly inhibit ADCC to the microfilariae (figure 1) at an

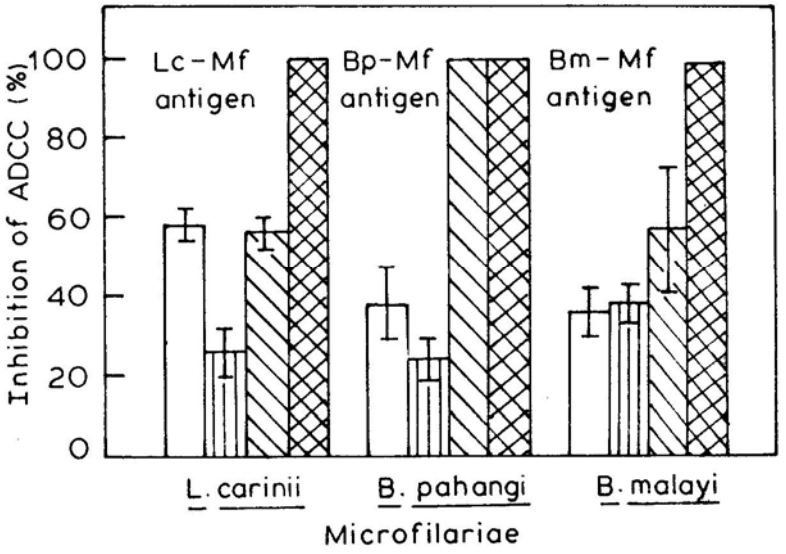

Figure 1. Inhibition of ADCC against microfilariae of different species of immune sera raised against *L. carinii,□; B. Pahangi,* ▥ *; B. malayi,* ▨ *;* and of sera from bancroftian elephantoid patients ▩ ; on absorption with microfilarial antigens.

antigen concentration of 5 μg/ml. The ADCC was completely blocked when the sera were treated with 25 μg of antigen, indicating the extensive sharing of antigens among these species. The low cytotoxicity of ELS was abolished on prior treatment with 5 μg of antigen.

Effect of antisera on circulating microfilariae of L. carinii

The effect of different antisera on circulating *L. carinii* microfilariae in *vivo* was studied by a passive transfer experiment described under 'methods'. An intraperitoneal injection of $1{\cdot}25 \times 10^6$ of *L. carinii* microfilariae in a 3-4 week old *Mastomys* resulted in a parasite count of about 200 Mf/10 μl of the peripheral blood (table 2). The microfilaremia was stabilised in the animals usually at day 3 when the animals were given intraperitoneally 1·5 ml of the immune sera. There was a rapid disappearance of the microfilariae from the circulation within 24 h (table 2) in all the groups except the one receiving fNRS. The parasites did not reappear till day 14 indicating that the immune sera killed the circulating Mf in *vivo.*

Table 2. Effect of heterologous immune sera on circulating microfilariae of *Litomosoides carinii* in *Mastomys natalensis.*

	Microfilaremia in 10 μl of blood ($x \pm$ S.D.)					
Group†	Day 1	Day 3	Treatment with	Day 4	Day 7	Day 14
1	110 ± 44	171 ± 53	Lc-Mf AIRS	0	0	0
2	80 ± 31	164 ± 52	Bp-Mf AIRS	$1 \pm 0{\cdot}9$	0	0
3	91 ± 23	188 ± 45	Bm-Mf AIRS	2 ± 1	0	0
4	193 ± 42	180 ± 39	ELS	0	0	0
5	78 ± 21	241 ± 58	fNRS	247 ± 60	273 ± 97	295 ± 100

† Each group consisted of 6 *Mastomys*.

Discussion

There is increasing evidence on sharing of antigens among filarial parasites. Neppart (1974) found cross reactions between *Onchocerca* Sp., *Dirofilaria immitis* and *L. carinii* in the closed hexagonal immunodiffusion assay. The antigens of *Setaria digitata* react with serum antibodies of *W. bancrofti* infected subjects in ELISA (Dissanaike and Ismail, 1980). Sharing of antigens between *L. carinii* and *W. bancrofti* has also been demonstrated (Rao *et al.,* 1980). Tandon *et al.* (1983), based on the presence of common antigens between *L. carinii, D. viteae, B. malayi* and *B. pahangi* parasites, studied the pattern of antibody titres in sera ofanimals infected with these parasites using *L. carinii* adult antigens. Similar cross reactivity of surface antigens has been demonstrated by Maizels *et al.* (1983) in *B. pahangi, B. malayi* and *B. timori.*

The present study supports the above observations on the sharing of antigens between *L. carinii, B. pahangi, B. malayi* and *W. bancrofti.* This conclusion is based on

the ability of *L. carinii* antigens to neutralize the ADCC activity of different antisera against the microfilarial species and the ability of the antisera raised against these parasites and of ELS in clearing circulating *L. carinii* microfilariae from *Mastomys.* The antisera directed against *L. carinii, B. pahangi* and *B. malayi* was more active in inducing ADCC to these parasites than ELS. This difference may be due to the high titre of the antibodies in immunized rodents when compared to the antibodies resulting from infection in patients (ELS).

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