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**Autor:** Mehta, K. / Subrahmanyam, D. / Sindhu, R.K.  
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Ciba-Geigy Research Centre, Goregaon East, Bombay 400 063, India

## **Immunogenicity of homogenates of the developmental stages of *Litomosoides carinii* in albino rats**

K. MEHTA<sup>1</sup>, D. SUBRAHMANYAM, R. K. SINDHU<sup>2</sup>

### **Summary**

Homogenates of different developmental stages of the filarial parasites, *L. carinii*, emulsified in Freund's complete adjuvant have been examined for their efficacy in conferring immunity to the infection in the albino rats. The results revealed that sonicated preparations of microfilariae and infective larvae induce high resistance to the infection in the animals. In contrast, soluble antigens of adults and sonicated homogenates of adult males were ineffective to induce such resistance. Some resistance seen when sonicated adult worms of both sexes were used as immunogens appears to be due to the microfilarial antigen present in the extracts.

*Key words:* *Litomosoides carinii*; immunization; homogenates; microfilariae; infective larvae; adults.

### **Introduction**

Albino rats infected with the filarial parasite *Litomosoides carinii* develop acquired resistance to the infection as revealed by the gradual disappearance of microfilariae from the peripheral blood (Ramakrishnan et al., 1962). However, adult worms continue to be alive and active in the pleural cavity of such animals leading to a condition of latency to the infection. At the onset of latency, IgE is detectable in the sera of the animals which promoted adhesion of macrophages and neutrophils to the microfilariae and infective larvae and caused cytotoxicity and death to the parasites (Mehta et al., 1980).

<sup>1</sup> Present address: Department of Pharmacology, Medical School, University of Texas, Houston, Texas, USA

<sup>2</sup> Present address: Department of Pharmacological Sciences, SUNY, Stony Brook, New York, USA

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Correspondence: Prof. D. Subrahmanyam, Ciba-Geigy Research Centre (Contribution number 63b), Goregaon East, Bombay 400063, India

Resistance to the infection could also be induced in normal animals by immunization with infective larvae attenuated by appropriate doses of radiation (Rao et al., 1977). However, Subrahmanyam and Bagai (1970) reported in a preliminary study that adult worm extracts did not confer protection against the infection. The present communication reports the results of a detailed study on the efficacy of the homogenates, suitably processed, of different developmental stages of the parasites such as microfilariae, infective larvae and adults in inducing resistance to the challenging infections.

## Materials and methods

*Maintenance of the infection.* Methods for the maintenance of the tropical mite vector, *Bdellonyssus bacoti*, for the infection of albino rats with *L. carinii* and monitoring of the infection by examination of the stained smears of the peripheral blood of the animals were as described elsewhere (Bagai et al., 1968).

*Isolation of developmental stages.* Microfilariae were isolated from the defibrinated blood of infected rats by the method of Subrahmanyam et al. (1978), using Ficoll-Hypaque of density 1.05. Infective larvae ( $L_3$ ) were collected by gently crushing the infective mites and separating the larvae from the mite debris under a microscope. Adult worms were removed from the pleural space of infected rats and rinsed in saline. The parasite material was stored in cold at  $-30^\circ\text{C}$  till use.

*Preparation of antigens and immunization.* Microfilariae and  $L_3$  were taken separately in a small volume of saline (PBS) of pH 7.2 and sonicated at 60 watts for 20 min at  $2-3^\circ\text{C}$ . The suspension was emulsified with an equal volume of Freund's complete adjuvant (FCA) and injected intramuscularly into 20-day-old albino rats. Eleven animals were used for immunization with microfilariae antigen and each animal received sonicated homogenate of  $1 \times 10^5$  microfilariae emulsified with FCA in two divided doses at an interval of 7 days.

Six animals were used for immunization with  $L_3$ -antigen and each animal received 240 sonicated  $L_3$  antigen emulsified in FCA in two divided doses at an interval of 7 days.

The soluble antigens were isolated from adult worms following the method of Bagai et al. (1968). Briefly the adult worms were ground twice with chilled ether in a pestle and mortar. The residue was then homogenized in PBS and centrifuged at 1000 g for 20 min in a table centrifuge. The supernatant fraction was dialyzed against PBS and the protein content was estimated. Six 20-day-old albino rats were immunized by intramuscular route with soluble antigens from adult worms emulsified in an equal volume of FCA. Each animal received 1.5 mg of worm protein in two divided doses at an interval of 8 days. Groups of 20-day-old albino rats were also immunized with sonicated homogenates of adult male worms emulsified in FCA or sonicated extracts of homogenates of adult worms of both sexes emulsified in FCA. For this purpose the worms were taken in PBS, homogenized and then sonicated at 60 watts for 20 min at  $2-3^\circ\text{C}$ . The protein content of the homogenates was determined. The suspension was emulsified with an equal volume of FCA. Six animals were used for immunization with each antigen. Each animal received worm material equivalent to 1.5 mg of protein in two divided doses at an interval of 8 days.

Four age-matched control animals for each experimental group received by intramuscular route equivalent amount of FCA emulsified in saline.

In most cases, animals of the control and experimental groups were challenged, 3-4 weeks after the last immunization, with about 50 live  $L_3$  by intramuscular route. At the end of the pre-patent period, the tail blood was examined at weekly intervals for the presence of microfilariae. At the end of the experiment, the animals were necropsied and the adult worms were recovered from the pleural cavity and counted.

## Results

The results on the ability of microfilariae homogenates of *L. carinii* in inducing resistance to the challenging infection in albino rats are presented in Table 1 (group I). It is apparent that the microfilariae antigen was highly effective in immunizing the animals against the filarial infection. There was no development of the  $L_3$  in the immunized animals except in two of the 11 animals. In these two, only 2 larvae developed to the adult stage out of about 55 injected. The pleural exudate of the animals, on examination, under microscope, had no microfilariae in them. The controls came down with the infection as seen from the microfilaremia and presence of adult worms and microfilariae in the pleural fluid.

Similar resistance to the infection was induced in the animals when they were immunized with infective larval homogenates (Table 1, group II). In general, the infective larvae used for challenge failed to develop in the  $L_3$ -immunized animals and no microfilariae were seen in the peripheral blood or in the pleural cavity of the animals.

No such resistance, however, was seen when the animals were immunized with the soluble adult worm protein antigens (Table 1, group III). Four of the

Table 1. Effects of various immunizations on microfilaremia and adult worm recovery from albino rats challenged with *L. carinii* infective larvae ( $50 \pm 5$   $L_3$  per animal)

Group	Immunization with	Number of patent/total animals	Peak microfilaremia (per 20 mm <sup>3</sup> blood) Geom. mean $\pm$ S.D.	Number of adult worms Arith. mean $\pm$ S.D.
I .....	$10^5$ sonicated microfilariae	0/11	0 (0/0)*	$0.4 \pm 0.8^*$
	controls	4/4	110 (24/495)	$6.3 \pm 2.4$
II .....	240 sonicated $L_3$	0/6	0 (0/0)*	$0.3 \pm 0.5^*$
	controls	4/4	182 (68/482)	$6.5 \pm 1.3$
III .....	soluble adult worm antigen (protein)	4/6	27 (2/362)	$5.8 \pm 4.2$
	controls	4/4	214 (165/278)	$6.5 \pm 1.0$
IV .....	adult worm homogenate	1/6	1 (0/3)*	$2.7 \pm 2.4^*$
	controls	4/4	120 (66/218)	$7.5 \pm 1.7$
V .....	adult male worm homogenate	6/6	120 (47/310)	$6.3 \pm 2.4$
	controls	4/4	170 (93/315)	$8.2 \pm 2.2$

\* t-test  $p < 0.001$

six experimental animals developed microfilaremia. All the animals had adult worms in their pleural cavity.

The failure of the adult worm soluble antigens in inducing resistance did not rule out the possibility of somatic insoluble antigens in conferring such a resistance. Alternately, the soluble antigens of the adult worms might not contain adequate levels of microfilarial antigens which were effective in immunizing against the infection (Table 1, group I). It was indeed found that the sediment of the adult worm homogenates after centrifugation did contain many intact microfilariae and embryos when examined under microscope. Therefore, in the next experiment the adult worm homogenates were sonicated to disintegrate the microfilariae and the total material emulsified in FCA was used for immunizing the animals. The data presented in Table 1 (group IV) revealed greater efficacy of the sonicated homogenates in conferring resistance to the infection. However, in 4 of the 6 experimental animals few of the infective larvae developed to adults.

The ability of the sonicated adult homogenates in inducing such resistance appeared to be due to the microfilariae antigen present in them but not due to the adult antigen per se as seen in the data of the next experiment (Table 1, group V). The sonicated adult male antigen was totally ineffective in inducing resistance to the infection in the animals.

## Discussion

Much of the earlier work suggested that dead filarial parasites were ineffective in inducing protection against homologous infections (MacFadzean, 1953; MacDonald and Scott, 1953; Krishnaswamy and Pattanayak, 1959). Non-living parasites were considered, in general, to be poor antigens in inducing immunity to helminthic infections (Terry, 1968).

Bagai and Subrahmanyam (1970) presented evidence that acquired resistance to filarial infection with *L. carinii* in albino rats was due to development of immune response directed against the microfilarial stage of the parasites. In these studies the authors could also note the resistance to develop in course of time by transplantation of adult worms of both sexes or females into the pleural cavity of the animals. No such resistance was seen to occur when adult males were used for transplantation. Similarly, female worms exhausted of microfilariae by serial passages in rats till they no longer caused microfilaremia failed to induce acquired resistance. However, repeated injections of live microfilariae, collected from the thoracic cavity of infected rats, into normal rats resulted in development of acquired resistance which led the authors to conclude that microfilariae were the immunizing agents. Wong (1964) showed that such repeated injections of live *Dirofilaria immitis* microfilariae into dogs resulted to a stage when transfused microfilariae disappeared quickly from the peripheral blood of the animals. Wegerhof and Wenk (1975) also observed that

live microfilariae of *L. carinii* have the ability to induce similar resistance in cotton rats to the infection.

Recent studies, however, indicate the possibility of conferring resistance to certain infections with parasite components. Rajasekariah et al. (1980) observed a high degree of protective effect in mice against *Taenia* infection on immunization with whole Oncospheres or a soluble fraction from the material. Haque et al. (1978) reported that immunization with crushed microfilariae or adult parasites caused reduction of microfilariae in hamsters infected with *Dipetalonema viteae*. Similar conclusions can be drawn from the work reported here that dead parasites could be effective in inducing resistance to filarial infection provided the appropriate developmental stage, processed suitably, is used as the immunogen. The ability of sonicated microfilariae and L<sub>3</sub> antigens in inducing resistance to the infection seen in the present studies suggest that microfilariae share certain protective antigens with L<sub>3</sub> stage of the parasites. The inability of the soluble adult worm or sonicated adult male antigens in inducing such a resistance implicate lack of appropriate antigens in them effective against challenge with L<sub>3</sub> stage of the parasite. The significant resistance seen in animals immunized with the sonicated adult worm homogenates could be due to the presence of microfilarial antigens in the extracts.

In a more recent study Storey and Mettias (1980) successfully immunized cotton rats, with homogenates of adult worms of both sexes. There was no development of microfilaremia in immunized animals although adult worms developed in the pleural cavity. In these experiments also, the resistance to the infection due to microfilariae antigens of the homogenates cannot be ruled out.

These data, therefore, warrant an intensive investigation on the isolation of the functional antigens from the larval stages which confer such an effective resistance to the filarial infection for possible use as vaccines.

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