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Somatic antigens from male and female adults of *Setaria cervi:* immune reactions in guinea pigs

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Abstract: Antigens obtained from adult female *Setaria cervi* were more protective than those of males against the development of microfilaremia in guinea pigs (transplanted with 10 *S. cervi*). In high dose levels (2 ml 500 µl/ml) significant resistance was induced by antigens from male (52% of protection) and female (62% of protection) worms. The antigens from female worms were effective (40% protection) at low dose level (2 ml 50 µl/ml) also.

Extensive studies on the development of immunity by somatic antigens obtained from nematodes have been reviewed by Lal *et al.*, (1985). In addition, studies on the somatic antigen-induced resistance in the host have produced contradictory results (Soulsby, 1963; Crandall and Arean, 1965; Lehnert, 1967; Guerrero and Silverman, 1969, 1971; Stromberg and Soulsby, 1977; Horii *et al.*, 1985). A careful screening of literature reveals that *Setaria cervi*, the most prevalent cattle filarial worm in the Meerut region, has remained untouched as regards the immunological aspects.

The antigenic activity of somatic extracts of male and female adults of *S. cervi* is reported here.

MATERIAL AND METHODS

The Host: I used infection free male guinea pigs of the Meerut strain, 90 days in age and weighing 560-600 g . The animals were housed 3-5 per cage with wire mesh floors, over sawdust at constant temperature and humidity. They received balanced commercially prepared food in pellet form and water *ad libitum.*

The Parasite: Adult *S. cervi:* Motile *S. cervi* were recovered from the peritoneal cavity of cattle slaughtered freshly in a local abattoir. They were washed thoroughly in physiological saline and the two sexes were separated.

Microfilariae (mf): Microfilariae were recovered fron the peripheral blood of the infected animals by density gradient centrifugation on Percoll in 0.25 M sucrose according to Chandrashekar *et al.*, (1984). The number of mf in a sample was estimated by dilution technique.

Preparation of Antigens: A known number of male and female worms was washed in distilled water and extruded in a Pressure Cell Extruder at 20,000 psi. This somatic antigen, obtained separately for the sexes, was freeze-dried and stored at 4°C until needed.

Adjuvant: 4% Sodium alginate in doses of 0.5 ml per animal.

Immunizing Schedule: Two injections of antigens administered intraperitoneally: the first on day 0 and the second on day 14.

Challenge Infection: 10 *S. cervi* adult females were transplanted into guinea pigs via laparotomy as described by Ansari (1964) on day 21 (i.e., 7 days after the second immunizing injection).

Experimental Design: The experiment involved 4 groups subdivided into 8 sub-groups

TABLE 1

Recovery of viable microfilariae (mf) from the peripheral blood circulation, 40 days after the challenge infection of guinea pigs with 10 adult female Setaria cervi *(after immunization)*

Group	Description of group	Antigens from male worms		Antigens from female worms	
		Recovery of mf (mf/ml of blood)		Recovery of mf (mf/ml of blood)	
		Mean (N=10) ± S.D.	% of protection (relative to control)	Mean (N=10) ± S.D.	% of protect (relative t control)
1	2 ml of 50 µl/ml somatic antigen + adjuvant	1,621 ± 278	24	1,337 ± 261[a]	40
2	2 ml of 500 µl/ml somatic antigen + adjuvant	1,016 ± 319[a]	52	834 ± 208[a]	62
3	Adjuvant alone (control)	2,137 ± 586	0	2,219 ± 612	0
4	Infection immunity control	198 ± 73[a]	91	223 ± 86[a]	90

[a] $P < 0.01$ (test vs. control); Student's t-test (Sokal and Rohlf, 1969).

(2 sub-groups A and B per group) of 10 animals each. Animals of the sub-groups A and B received antigens obtained from male and female worms respectively.

Group 1: The animals received 2 ml of 50 µl/ml of lyophilized somatic antigens in each injection.

Group 2: The animals received 2 ml of 500 µl/ml of lyophilized somatic antigens in each injection.

Group 3 (Control): The animals received adjuvant alone in two injections.

Group 4 (Active immunization control): The animals used were transplanted with 10 female worms 240 days before the challenge infection. In a separate experiment it was predetermined that the mf are cleared from the peripheral blood circulation of the animals implanted with 10 female worms, by the day 240 after the worm implantation.

For the collection of mf, the blood samples were taken on day 40 after the challenge infection. The peak microfilaremia was predetermined the day 40 after the worm transplantation, in a separate experiment.

RESULTS

Results are summarized in Table 1. Although the lyophilized somatic antigens obtained from the male worms reduced ($P < 0.01$) the microfilaremia at higher dose (500 µl/ml) only, those from the female worms did so significantly ($P < 0.01$) at both doses (50 and 500 µl/ml). Infection immunity control group animals offered maximum resistance (90% of protection).

DISCUSSION

A number of investigators have tried somatic antigens obtained from nematode stages moulted in the host and recovered from it to induce resistance against the respective nematode infections. They have obtained contradictory results in their efforts (Lal *et al.*, 1985). Present studies reveal that the lyophilized somatic antigens from the two sexes of *S. cervi* are potent enough (at high dose levels, 500 µl/ml; 2 ml: females at dose 50 µl/ml; 2 ml, also) to induce resistance into the host. The degree of resistance, however, is not that which may be expected as required to eliminate completely the mf from the host, or to protect it from the development of any microfilaremia. Results with control group animals support a number of previous reports (Levine, 1968): the repeated infections result in development of resistance in the host.

Studying *Ascaris suum,* Guerrero and Silverman (1969) obtained similar results and concluded that somatic antigens from third-stage larvae induce a limited protection to infection

in mice. Earlier, Soulsby (1963) reported a 92.3% protection in guinea pigs when injected with lyophilized somatic antigens of the same kind used here. He, however, obtained variable results later on; he found a 63% and 82% resistance to infections with embryonated eggs of *A. suum* using fresh antigens. This induced resistance was previously suggested by Soulsby (1958) to be due to the release of functional antigens when the larvae were disrupted.

It can be safely concluded that lyophilized somatic antigens obtained from the two sexes of *S. cervi* may be used in dose level of 2 ml of 500 µl/ml of antigen, to induce partial protective immunity in guinea pigs against the filarial worm.

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RESUMEN

Los antígenos de *Setaria cervi* hembra son más eficaces que los de macho para evitar el desarrollo de microfilaremia en cobayos (10 parásitos por individuo). Con dosis altas (2 ml 500 µl/ml) se obtuvo resistencia significativa a partir de antígenos de parásitos machos (52% de protección) y hembras (62%). Los antígenos de hembra también protegieron (40%) con dosis bajas (2 ml 50 µl/ml).

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