DETECTION OF HUMORAL IMMUNE RESPONSE TO *GNATHOSTOMA SPINIGERUM* IN MICE

MALINEE ANANTAPHRUTI, JITRA WAIKAGUL, SUWANNEE NITHI-UTHAI*, SOMCHIT PUBAMPEN and WICHIT ROJEKITTIKHUN

Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, and *Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

INTRODUCTION

Diagnosis of gnathostomiasis is currently based on many criteria: clinical symptoms of intermittant swelling, itching and pain, previous history of eating raw or half-cooked meat, eosinophilia, skin test, and discovery of the worm *Gnathostoma spinigerum* (Miyazaki, 1966; Daengsvang, 1980). A number of serological tests have also been applied, however, due to the non-specificity and insensitivity of the tests, the results have not been satisfactory (Punyagupta and Pacheco, 1961; Kasemsuth *et al.*, 1981; Suntharasamai *et al.*, 1985). Precipitin test has been shown to be highly sensitive and specific for the diagnosis of gnathostomiasis (Yamaguchi, 1952; Furuno, 1959). The objective of this study was to compare the humoral immune responses to early and advanced third stage larvae of *Gnathostoma spinigerum* in mice using Ouchterlony gel diffusion test in relation to worm count. *G. spinigerum* antigen and antisera were also tested for cross-reaction with antisera and antigens of other helminths.

MATERIALS AND METHODS

Experimental animals: One hundred and eighty helminth-free Swiss mice, 2 month-old of either sex, were divided into 3 groups each with 60 animals. Mice in the first group were each allowed to feed on infected cyclops containing 30 early third stage larvae (EL3) of *G. spinigerum*. In the second group, mice were each orally infected with 3 advanced third stage larvae (AL3), and mice in the third group were not infected and used as control group. Five mice in each group were bled at weekly intervals from retro-orbital plexus, sera were collected and kept at –20°C until used. The animal was sacrificed and examined for *G. spinigerum* advanced third stage larvae. The larvae recovered from the liver and muscle of infected mice were counted and the percentage recovery was determined.

Parasites: Two types of *Gnathostoma spinigerum* larvae, early third stage larvae (EL3) and advanced third stage larvae (AL3) were obtained from infected cyclops and infected mice respectively.

Antigen: Crude soluble antigen was prepared from *G. spinigerum* AL3(Gs antigen). The larvae were washed several times in 0.85% normal saline and 2-3 times in 0.01 M PBS pH 7.2. They were then homogenized in a glass tissue grinder on ice for 20-30 min. The suspension was spun for 30 min at 10,000xg, 4°C and the pellet was discarded. The protein content of the antigen, determined by Lowry method (Lowry *et. al.*, 1951) was 6.78 mg/ml. Antigens of other helminths were also prepared as mentioned above: *Angiostrongylus cantonensis* adult, *Paragonimus siamensis* adult, *Trichinella spiralis* larvae, *Opisthorchis viverrini* adult and *Ancylostoma ceylanicum*, larvae and the protein contents of those antigens were 8.3 mg/ml, 1.16 mg/ml, 1.44 mg/ml, 3.4 mg/ml and 2.8 mg/ml respectively.

Serological test: Gel diffusion test was performed according to Ouchterlony (1949), using 1% agarose in 0.05 M veronal buffer pH 8.2. The antigen-antibody reaction was allowed to develop at 4°C for 24 hr. The slides were then washed in normal saline, distilled water, dried and stained with amino black 12B. The test was done on: (a) Gs. antigen and sera of experimental mice of every group, (b) positive sera of infected mice and antigens of *Angiostrongylus cantonensis*, *Paragonimus siamensis*, *Trichinella spiralis*, *Opisthorchis viverrini* and *Ancylostoma ceylanicum*, and (c) Gs antigen and sera of angiostrongyliasis in rats, paragonimiasis in bandicoots, trichinellosis in mice, opisthorchiasis in hamsters and hookworm infection in cats.

RESULTS

G. spinigerum AL3 recovered from mice infected with EL3 is shown in Table 1. In the first two weeks most of the larvae were in the liver, and few in the muscle. The larvae found in the muscle increased gradually, at wk 4, half of the larvae were in the muscle and at wk 12 most of the larvae were found in the muscle. Larvae encysted at wk 5, and completely encysted in wk 10. The number of *G. spinigerum* AL3 recovered from mice infected with AL3 is shown in Table 2. From second week, most of the larvae were found in the muscle, encystation of larvae began in wk 3 and completed in wk 5. Encystation of the larvae of mice infected with AL3 started 2 weeks earlier and completed 5 weeks earlier than the mice infected with EL3.

The seropositive rates of mice infected with EL3 and AL3 by gel diffusion are shown in Fig. 1. The EL3 infected group became positive 3 weeks after infection, the highest percentage (100%) was demonstrated from wk 4, the positive percentage started to decrease from wk 8 and become completely negative at week 11. The second group infected with AL3 became positive in the

Table 1

Gnathostoma spinigerum advanced third stage larvae recovered from liver and muscle of mice infected with early third stage larvae in cyclops.

Duration (wk)	%Worm recovery						% total recovery
	Liver		Muscle		Total		
	Larvae	Encysted larvae	Larvae	Encysted larvae	Larvae	Encysted larvae	
1	5.3	-	4	-	9.3	-	9.3
2	63.3	-	1.3	-	64.6	-	64.6
3	58.0	-	7.3	-	65.3	-	65.3
4	24.0	-	21.3	-	45.3	-	45.3
5	10.7	4.0	36.0	1.3	46.7	5.3	52.0
6	6.0	5.3	22.0	19.3	28.0	24.7	52.7
7	-	12.7	-	34.0	-	46.7	46.7
8	-	10.0	0.7	42.0	0.7	52.0	52.0
9	-	4.0	0.7	45.3	0.7	49.3	49.3
10	-	3.3	-	43.3	-	46.7	46.7
11	-	4.0	-	19.3	-	23.3	23.3
12	-	0.7	-	30.7	-	31.3	31.3

Table 2

Gnathostoma spinigerum advanced third stage larvae recovered from liver and muscle of mice infected with advanced third stage larvae.

Duration (wk)	%Worm recovery						
	Liver		Muscle		Total		% total recovery
	Larvae	Encysted larvae	Larvae	Encysted larvae	Larvae	Encysted larvae	
1	26.7	-	33.3	-	60	-	60
2	6.7	-	53.3	-	60	-	60
3	-	-	20.0	6.7	20.0	6.7	26.7
4	6.7	-	20.0	46.7	26.7	46.7	73.3
5	-	-	-	26.7	-	26.7	26.7
6	-	6.7	-	20.0	-	26.7	26.7
7	-	-	-	13.3	-	13.3	13.3
8	-	-	-	33.3	-	33.3	33.3

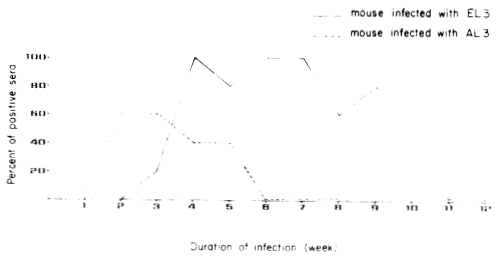

Fig. 1—Percent of positive sera of mice infected with early third stage larvae (EL3) and advanced third stage larvae (AL3) in each week from 1 to 12 weeks.

second week with the highest 60% positive sera, percentage of positive sera decreased in the fourth week and become completely negative at week 6. It was observed that by gel diffusion the positive sera were detected in mice when a number of larvae were presented in the muscle and one week after completed encystation of larvae, the result was negative. The response of mice to EL3 rose slower than the response of mice to AL3 but higher and last longer.

Specificity of gel diffusion was confirmed by the failure of precipitin band to appear on the *G. spinigerum* antisera against the antigens of *A. cantonensis*, *P. siamensis*, *T. spiralis*, *O. viverrini* and *A. ceylanicum* (Fig. 2). However, the poorly developed precipitin bands were observed on the Gs antigen against angiostrongyliasis rat antisera and paragonimiasis bandicoot antisera (Fig. 3).

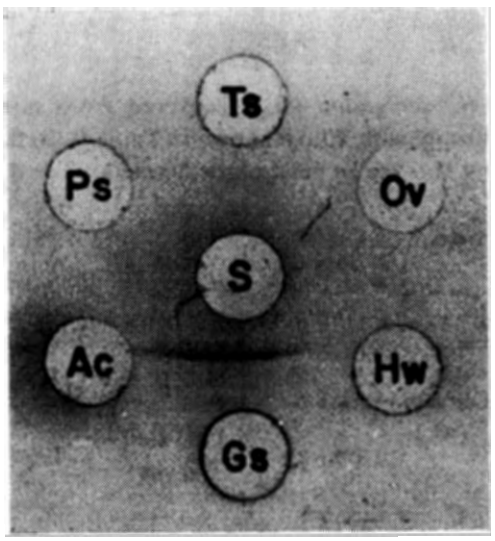

Fig. 2—The precipitin band on the *G. spinigerum* antiserum of EL3 infected mouse 5 wk after infection(s) against Gs antigen (Gs). No band was observed on this antiserum against the antigens of *A. cantonensis* (Ac), *P. siamensis* (Ps), *T. spiralis* (Ts), *O. viverrini* (Ov) and *A. ceylanicum* (Hw).

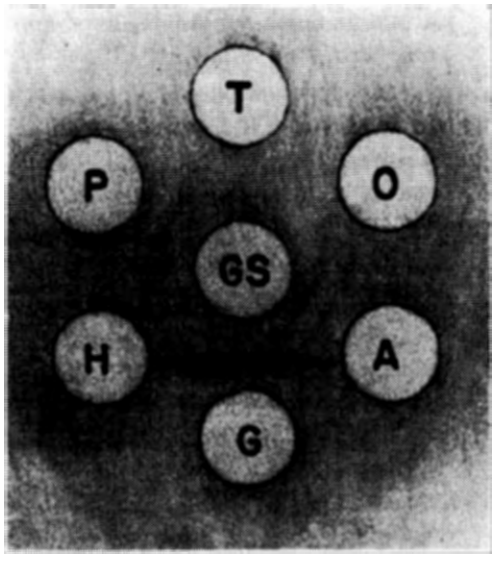

Fig. 3—The precipitin bands on the Gs antigen (Gs) against EL3 infected mouse antiserum (G), *A. cantonensis* rat antiserum (A) and *P. siamensis* bandicoot antiserum (P). No reaction was observed on this antigen against *A. ceylanicum* cat antiserum (H), *T. spiralis* mouse antiserum (T) and *O. viverrini* hamster antiserum (O).

DISCUSSION

The larvae presented in the muscle correlate with the number of positive sera. Sera become positive when a considerable number of larvae are found in muscle and one week after all larvae encysted, sera become negative. The size of infected larvae seems to correlate with the duration of first detection of antibodies. AL3 are larger than EL3 and the antibody appeared earlier in mice infected AL3 than that with EL3. This may due to more amount of toxin or metabolic waste product produced by AL3 than that by EL3.

Punyagupta and Pacheco (1961) reported the detection of antibodies by indirect hemagglutination test in rabbits infected with *G. procyonis*. The time interval between infection and the first détection of antibodies and the rate of antibody production depended on the number of worms infected. From our experiment, it showed that the interval of infection and the initial antibody detection depended more on the size of the worms than the number of infected worms, but the duration of antibody production depended on the number of infected worms more than the size of the worms itself. The percentage of positive sera for Ouchterlony related to the number of worms infected to the hosts.

Yamaguchi (1952) has shown the precipitin ring reaction on the adult worm antigen of gnathostome from cats and weasels, and sera of human gnathostomiasis, these sera gave negative results with *Spirometra mansoni*, *Clonorchis sinensis* and *Schistosoma japonicum*. Furuno (1959) also demonstrated the precipitin reaction on various extract of antigens and rabbit sera infected with *G. spinigerum*, no cross reaction was observed on these sera and the extract of roundworm from pig and *Paragonimus westermani*. From our study, no cross reaction was observed on the positive mice infected sera and the antigens of *A. cantonensis*, *P. siamensis*, *T. spiralis*, *O. viverrini* and *A. ceylanicum*. However, cross reaction was observed on *G. spinigerum* antigen and angiostrongyliasis sera in rats and paragonimiasis sera in bandicoots.

SUMMARY

The humoral immune response to early third stage larvae (EL3) and advanced third stage larvae (AL3) of *Gnathostoma spinigerum* infection was studied in mice by Ouchterlony gel diffusion technique. The antibodies was detected at week 3 in mice infected with EL3 and remained up to week 10 after infection. Highest positive sample of sera were demonstrated at week 4 to week 7. Similar results were obtained from AL3 infected sera except the antibodies was found and disappeared earlier (week 2 to week 6). *G. spinigerum* larvae recovery from mice in both groups showed that the number of advanced third stage larvae located in muscle correlated to

the peak of positive sera. No cross reaction was observed on positive sera of *G. spinigerum* and antigens of *A. cantonensis*, *P. siamensis*, *T. spiralis*, *O. viverrini* and *A. ceylanicum*. Cross reaction was shown on the *G. spinigerum* antigen against rat sera with angiostrongyliasis and bandicoot sera with paragonimiasis.

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