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Specific IgG antibody responses in experimental cat metagonimiasis*

Seung-Yull Cho, Suk-Il Kim and Shin-Yong Kang
Department of Parasitology, College of Medicine, Chung-Ang University, Seoul 151, Korea

INTRODUCTION

In metagonimiasis the pathologic lesions are made in small intestinal mucosa. The main findings were villous changes such as thickening, fusion and shortening, stromal infiltrations of inflammatory cells, edema and vascular ectasia in the mucosal stroma and intraepithelial cell infiltrations (Chai, 1979; Lee *et al.*, 1981; Kang *et al.*, 1983; Rho *et al.*, 1984). These findings reached their peak in 5-28 days after infection. Then it returned slowly to normal unless reinfection occurred (Lee *et al.*, 1981; Kang *et al.*, 1983). Among the above, lymphocytic infiltration to mucosal stroma as well as mucosal epithelium suggested that certain immunologic responses were taken place at the niche to infected parasites.

Whatever the nature of infiltrated lymphocytes in mucosal stroma or epithelium, either B- or T-lymphocytes, serologic studies in metagonimiasis should have been done. They may make understanding of intestinal lesions clearer and provide information whether serologic diagnosis of human as well as animal metagonimiasis would be possible. In experimental metagonimiasis of rabbits, Yeo *et al.* (1985) found elevated specific antibody (immunoglobulin class not specified) levels during 1-21 weeks after infection when measured by enzyme-linked immunosorbent assay (ELISA).

In this study, we observed the specific IgG antibody responses in experimental metagonimiasis of cats to characterize the usefulness of serologic diagnosis in this disease.

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MATERIALS AND METHODS

1. Antigens

Two different antigens, metacercariae and adult worms of *Metagonimus yokogawai*, were prepared. About 1.11 g of metacercariae (about 500,000 when estimated using a Wintrobe tube) were collected from naturally infected sweetfish (*Plecoglossus altivelis*) by peptic digestion. It was purified by washings and removing particles of fish muscle and scales under a dissecting microscope. It was ground with teflon-coated tissue homogenizer in 0.5ml of physiologic saline at 4°C for 5 minutes. Cyst walls were also comminuted. Adding 20ml of saline, the tissue emulsion was shaken for 2 hours at room temperature. Then it was placed at 4°C for additional 22 hours. It was centrifuged at 4°C by 10,000 *g* for 30 minutes. Supernatant was used as metacercarial antigen.

About 1.09 g of 4-week old adults of *M. yokogawai* was collected from an experimentally infected dog. It was washed with saline until no more host tissue particles and mucin were recognized. Saline extract was prepared as the above and it was regarded as adult antigen. By homogenization procedure, eggs in adult worms were not beaten.

Protein contents as measured by the method of Lowry *et al.* (1951) were 3.03mg/ml for the metacercarial antigen and 2.46mg/ml for the adult antigen.

2. Experimental infection and serum collection

A total of 25 cats were infected with metacercariae collected from sweetfish. The fish were purchased in Jangheung Gun, Cholla Nam Do. Each cat was challenged with 10,000 or 80,000 metacercariae (Table 1) and was killed on 3, 7, 10 days and 2, 4, 8 and 12 weeks after infection respectively. Just before the sacrifice, blood was drawn by cardiac puncture. Therefore, only one serum was available for each cat.

The number of infected worms was counted in each cat. Small intestine, from duodenum to ileum, was examined in saline. Sera from 3 non-infected cats were used as control.

3. Enzyme-linked immunosorbent assay

The technique of McLaren *et al.* (1978) was followed. Chequerboard titrations were done with different dilutions of antigens, infected and non-infected cat sera and peroxidase-conjugated anti-cat IgG (heavy and light chain specific, Cappel, USA). Actual test condition adopted was 3.0μg/ml of antigens, 1 : 100 diluted sera and 1 : 1,000 diluted conjugate. At the final stage of ELISA, substrate (made of 99ml of distilled water, 1ml of 1% *o*-phenylene diamine and 10μl of 30% H_2O_2) was reacted for 30 min. The color was read for the absorbance at 492nm with Gilford spectrophotometer.

RESULTS

As shown in Table 1 and Fig. 1, sera from 3 non-infected cats showed low absorbance of specific IgG antibody levels (0.087, 0.067, 0.047 for the metacercarial antigen and 0.046, 0.074 and 0.048 for the adult antigen). Antibody levels began to rise after 7 days of infection, reached peak for 2-4 weeks and made plateau thereafter. Later than 10 days of infection, IgG antibody levels to the adult antigen in infected cat sera were higher (mean and standard devia-

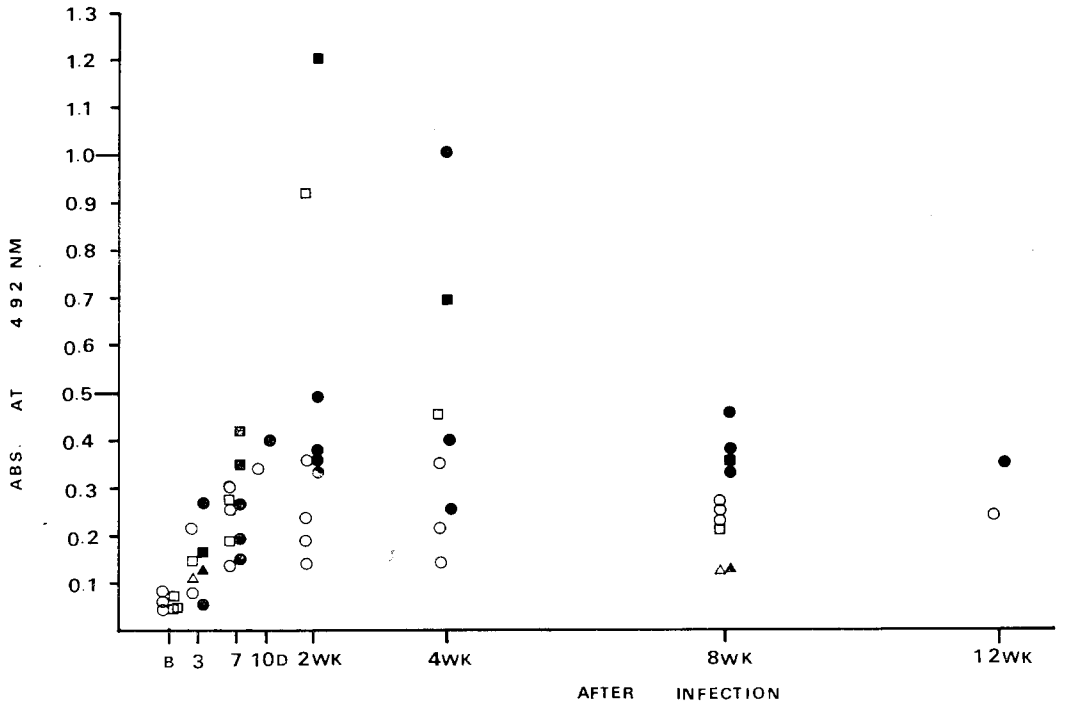

Fig. 1. Specific IgG antibody levels in individual cat as measured by ELISA. Open triangle, circle and rectangle (△, ○, □) mean antibody levels to metacercarial antigen while closed ones (▲, ●, ■) mean those to adult antigen. Triangles (△, ▲): cats infected with less than 1,000, circles(○, ●): cats infected with 1,000~5,000, rectangles (□, ■): cats infected with more than 5,000 adults. Markings (○, □) of B (in control cats) represents abs. to metacercarial (○) or adult (□) antigens.

Table 1. Specific IgG antibody levels as measured by ELISA in 25 experimental cats infected with different doses of *Metagonimus yokogawai*

Days(weeks) after exp. infection	Doses of metacercarial challenge	No. of cats	Mean No. of adults recovered	Mean abs. to	
				metacercarial antigen	adult antigen
Control	0	3	0	0.065	0.056
3 days	10,000	1	105	0.109	0.127
	10,000	2	2,918	0.150	0.164
	80,000	1	20,572	0.149	0.165
7 days	10,000	3	2,280	0.223	0.210
	80,000	2	41,609	0.232	0.383
10 days	10,000	1	3,496	0.345	0.397
2 weeks	10,000	4	2,240	0.238	0.395
	80,000	1	7,342	0.935	1.206
4 weeks	10,000	3	2,459	0.239	0.556
	80,000	1	9,353	0.455	0.696
8 weeks	10,000	1	153	0.130	0.131
	10,000	3	2,608	0.264	0.395
	80,000	1	10,846	0.221	0.358
12 weeks	10,000	1	4,084	0.247	0.363

tion, 0.474 ± 0.276), without exception, than to the metacercarial antigen (0.299 ± 0.190).

Mean and standard deviation of IgG antibody to the adult antigen in 12 cats infected with 1,000~5,000 adults were 0.433 ± 0.191 during 10 days~12 weeks. But those in 3 heavily infected cats (with more than 5,000 adults) showed absorbance 0.753 ± 0.426 to the adult antigen. A cat infected with 153 adults showed very low absorbance, 0.13 to both antigens.

DISCUSSION

Metagonimus yokogawai, like other intestinal trematodes, shares two characters of tissue invader and lumen dweller. As Rho *et al.* (1984) described, adult worms invade crypto-villous junction of small intestinal mucosa and destroy mechanically the maturing enterccytes and goblet cells. Tissue defects are made there. It caused deformation of villi or fusion with nearby ones. In this connection, Kim *et al.* (1985) reported that the height of small intestinal villi was related inversely with worm number in metagonimiasis. At present, however, it is not clear whether this mechanical destruction is the only cause of the intestinal lesions. In addition to the villous changes, infiltration of inflammatory cells to mucosal stroma and of lymphocytes to villous epithelium persisted (Lee *et al.*, 1981). The presense of these immunologic effector mechanisms suggested strongly that serologic responses were elicited in this infection.

Because our serum samples were not collected serially from each cat, the number of examined sera was small in total. However, specific IgG antibody levels were significantly high after 10 days of infection. Our data suggested that the levels of IgG antibody were affected by the number of parasites in small intestine. Cats infected with only hundreds of adults showed far lower level of the antibody when compared with cats infected with thousands in the same infection period. Extrapolation of this relation between numbers and antibody levels suggested lowering of antibody levels after natural cure or treatment with drugs. As in clonorchiasis (Han *et al.*, 1986), the serologic test in lightly infected metagonimiasis may be less sensitive. But the present study confirmed that serological diagnosis of human metagonimiasis is possible in the early clinical stages of the infection.

Paucity of serologic study in metagonimiasis hitherto may be partly due to a difficulty in obtaining non-contaminated worms. It needs long labor. Using these antigens further studies are necessary such as antigen analysis and its relation with intestinal lesions.

SUMMARY

In order to observe the feasibility of serologic diagnosis of metagonimiasis, saline extracts of metacercariae and 4-week old adults were prepared. Sera from 25 experimentally infected cats were collected from 3 days to 12 weeks after infection. Their levels of specific IgG antibody were measured by ELISA together with 3 sera from non-infected cats.

Specific IgG antibody levels began to rise in 7 days after infection, reached their peak in 2–4 weeks and made a plateau thereafter. Cats infected with hundreds of adult worms showed minimal rise of the antibody level. Adult antigen was superior to metacercarial antigen in detecting the specific IgG antibody.

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=국문초록=

실험적 요꼬가와흡충증에서의 특이 IgG 항체 반응

중앙대학교 의과대학 기생충학교실
조 승 열·김 석 일·강 신 영

요꼬가와흡충을 고양이 등에 실험적으로 감염시키면 그 기생부위인 소장의 점막에는 융모의 위축, 융합 등의 병적변화가 일정기간 나타나며 점막기질(stroma)에는 심한 염증세포의 침윤이 일어난다. 이상의 병리학적 소견을 기초로 생각할 때 요꼬가와흡충 감염 경과중 특이 항체반응이 나타날 것으로 가정할 수 있다. 이 연구에서는 요꼬가와흡충의 피낭유충을 10,000개 및 80,000개씩 감염시킨 고양이 25마리에서의 혈청내 특이 IgG 항체가를 면역효소측정법으로 조사하였다. 자연감염된 온어에서 분리한 피낭유충과 실험감염 개의 소장에서 4주일후 분리한 성충의 생리식염수 추출액을 항원으로 사용하였다. 그리고 감염된 충체의 수를 부검때에 세어 실제 감염량으로 하였다.

특이 IgG 항체가는 대조군 혈청에 비하여 감염 7일째부터 상승하기 시작하였으며 감염후 2~4주일에 최고치에 도달하였다가 그 이후 일정한 수준을 유지하였다. 특이 IgG 항체는 피낭유충 항원보다는 성충 항원에 대하여 더 높게 반응하였다. 그리고 감염충체수가 적은 고양이의 혈청내 IgG 항체가는 같은 감염기간의 대량감염 고양이에 비하여 낮았다.