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# IgA response in mice infected with *Neodiplostomum seoulensis*

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**Abstract:** To observe the production of IgA in Balb/c mice with neodiplostomiasis, 20 mice were infected with each 200 metacercariae of *Neodiplostomum seoulenis*. Sera and the duodenums were obtained 3, 7, 14, 28 days post-infection (PI) from five mice each group. *Neodiplostomum* specific IgA in serum by the enzyme-linked immunosorbent assay increased from 7 days PI and persisted till 28 days PI. Immunohistochemistry for IgA was done with sections of the duodenum. The IgA-positive reaction was generally seen in the lamina propria and submucosa. Some of epithelial cells were positive at 7 and 14 days PI. The present finding showed that *Neodiplostomun* specific IgA antibody increased in serum and that there was local reaction of IgA in the mucosa and submucosa of the duodenum but not directly related with worm expulsion.

**Key words:** *Neodiplostomum seoulensis*, mouse, IgA, enzyme-linked immnunosorbent assay, immunohistochemistry

## INTRODUCTION

*Neodiplostomum seoulensis*, Hong and Shoop, 1994 (old name: *Fibricola seoulensis*) has been known as one of the endemic human intestinal trematodes from 1982 in Korea (Seo *et al.*, 1982; Hong *et al.*, 1984 and 1986, Huh *et al.*, 1994). *N. seoulensis* entraps the villi of the intestinal mucosa and can cause diarrhea, and intestinal bleeding in human and experimental mice. After the infection of four weeks, over 90% of infected worms were expelled from experimental rats (Hong *et al.*, 1982). During the process of worm expulsion, there were known some kinds of immune reaction. Acute gastrointestinal symptoms in the first human case supports the strong immune reaction of the host to worms.

The number of mast cells of intestinal villi was found to increase from seven days post-infection (PI) and reached at a peak 21 days PI, after then, decreased (Kho *et al.*, 1990). The worm recovery rate of re-infection decreased significantly than that of the first infection (Yu *et al.*, 1995). *N. seoulensis* specific IgG reached at peak 10 days PI in experimental rats (Kho, 1992). Of many immunological components, we tried to trace the role of IgA during the process of worm expulsion. The IgA is well-known as primary immunoglobulin for the protection in the intestinal environment. We measured *N. seoulensis* specific IgA titer in sera by enzyme linked immunosorbent assay

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(ELISA) and also observed the IgA containing cells in the intestinal wall from experimentally infected mice.

## MATERIALS AND METHODS

### Materials

European glass snakes, *Rhabdophis tigrina*, were purchased in Chunchon, Korea. The snakes were peeled out and digested for two hours in 0.6% pepsin in 1% HCl solution at 37°C. The metacercariae were collected under the stereoscope. Twenty Balb/C mice (age of 4 weeks-6 weeks, regardless of sex) reared in the Animal Husbandary of Hallym University were infected *per os* each with 200 metacercariae of *N. seoulensis*. They were reared in the conventional room. Five mice were sacrificed by cervix cutting 3, 7, 14, and 28 days PI. Their sera were separated and stored at -70°C. The duodenum was fixed in 10% buffered formalin (pH 7.2). The worms collected from the duodenum of mice were homogenized in equal volume of 0.01M PBS (pH 7.4) at 4°C. The homogenates were centrifuged at 10,000 g, 4°C, and the supernatant was used as worm extract (antigen). Five normal mice reared at the same room for the experiment period were used the control.

### Measurement of *N. seoulensis* specific IgA in serum by enzyme linked immunosorbent assay (ELISA)

The worm extract was diluted to a concentration of 10 μg/ml in carbonate buffer (pH 9.6) and coated in EIA plates (Costar, USA) at 4°C overnight. The mouse sera were diluted to 1:100 in PBS/tween 20 (pH 7.4) and dispensed to the plate for 2 hours incubation at 37°C. Horse-raddish-peroxidase conjugated goat anti-mouse IgA (Sigma, USA) was diluted to 1:10,000 and reacted for 2 hours at 37°C. Colorization was made by adding 0.01% *o*-phenylenediamine in 0.03% $H_2O_2$. The reaction was stopped in 30 minutes by adding 8 M $H_2SO_4$. The absorbance was measured by an ELISA reader at 492 nm (MR700, Dynatech Laboratory Inc.).

### Observation of IgA in a duodenal mucosa by enzyme immunohistochemistry

Immediately after the sacrifice of the mice, the samples were taken from the duodenum and fixed in 10% neutral buffered formalin (pH 7.2). After fixation, the specimens were dehydrated in alcohol, cleared in xylene and embedded in paraffin. Sections were cut in 5-6 μm and stained immunohistochemically for IgA. In brief, the sections were treated in 0.5% periodic acid solution to remove the endogeneous peroxidase and followed in 10% normal goat serum to prevent the non-specific reaction. Sections were incubated with rabbit anti-mouse IgA for 48 hours at 4°C. After that, the sections were incubated in biotin-conjugated anti-rabbit IgG for 24 hours at 4°C, followed by conjugated avidin-biotin-complex for 24 hours at 4°C. The immunoreaction was revealed by adding diaminobenzidine. The sections were counterstained with Meyer's hematoxylin and observed with bright field light microscope (Axiophot, Zeiss).

## RESULTS

### Titer of anti-*N. seoulensis* specific IgA antibody

Titer of anti-*N. seoulensis* specific IgA antibody was expressed as an absorbance. Mean and standard deviation of each group was as followings: Control 0.1444 ± 0.017494, three days PI 0.1255 ± 0.030627, seven days PI 0.1757 ± 0.039571, 14 days PI 0.2041 ± 0. 097382, and 28 days PI 0.2733 ± 0.1257 (Fig. 1).

### Observation of IgA in the duodenal mucosa

In the control mouse, the villi/crypts ratio (V/C ratio) was 3:1-4:1. There was a IgA-positive reaction in the mucosal lamina propria and submucosa. None of the epithelial cells was stained (Figs. 2A & 2B). At three days PI, V/C ratio was similar with that of the control. There was neither villous atrophy nor crypt hyperplasia. The IgA-positive reaction was obeserved in the submucosa and the lamina propria between crypts. The lamina propria of

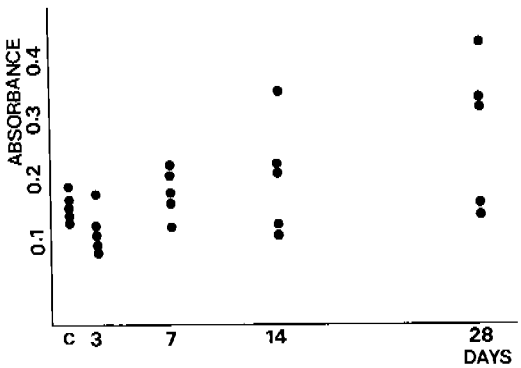


**Fig. 1.** *Neodiplostomum seoulensis* specific IgA in mice sera ater infection. At 28 days PI, the absorbance was significantly increased in comparison to that of control (*t*-test, $p < 0.05$).

villi showed weak reaction. Epithelial cells were not positive to IgA antisera. At seven days PI, V/C ratio became about 1:1. Crypt hyperlasia was seen as inflammatory findings. Not only the lamina propria of the villi but also some epithelial cells were positive to IgA antisera. The IgA-positive reaction was strong in the lamina propria of the villi and weak in other region (Figs. 2C and 2D). At 14 days PI, V/C ratio was reversed to 1:1-1:2. IgA-positive reaction was apparent in the lamina propria of the villi and between the bases of crypts. A few epithelial cells were also positive to IgA antisera. At 28 days PI, V/C ratio was 1:1- 1:2. Increased number of goblet cells was seen. The lamina propria and submucosa were widely and intermittently positive to IgA antisera. There were few IgA positive epithelial cells (Figs. 2E and 2F).

## DISCUSSION

In this study, we checked circulating IgA in sera and secretary IgA in the duodenal mucosa and submucosa. We could find continuous increase of serum IgA from three days PI to 28 days PI. Secretion of IgA, one of the local immune response of the mouse inestine to *N. seoulensis* was also seen in the villous epithelial cells. The secretary IgA in the mucosa was not specific to *N. seoulensis* but were non-specific. In every section, intercellular fluid was also stained. The stained fluid was believed to contain IgA. According to the fact that the epithelial cell was stained at 7 days PI, we could infer remarkable secretion of IgA into the intestinal lumen.

Increase of the circulating specific IgA was reported in mice infected with *Echinostoma caproni* (Agger *et al.*, 1993), *Giardia muris*, and in rats with *Trichinella spiralis* (Van Loveren, 1988). In mice infected with *E. caproni*, an intestinal trematode, significantly high level of IgA was measured in serum or in the lumen of the small intestine from 28 days PI. In rats infected with *T. spiralis*, the increased IgA response in serum and intestinal mucosa coincided with the expulsion of worms. Decline of the infection of *G. lamblia* was also concomitant with the increase of the lamina propria IgA containing cells (Vanayake *et al.*, 1991). An IgA monoclonal anti-*Entamoeba histolytica* antibodies was observed to inhibit adherence of trophozoites (Leyva *et al.*, 1992). In *Eimeria falciformis* infected mice, IgA containing lymphocytes accumulated in the apical portion of the lamina propira (Nash and Speer, 1988). A local IgA response against *G. muris* trophozoites in the mouse intestine and anti-trophozoite IgA may contribute to the clearance of *G. muris* from mice (Heyworth, 1989).

The present result also demonstrated the increase of circulating specific IgA in serum and the presence of local secretory IgA in the mucosal epithelium in the intestine after infection with *N. seoulensis*. There was a discrepancy between the increase of circulating IgA and secretory IgA in the mucosa. This is different with that of the *G. muris* experiment (Heyworth, 1989). It is understandable since the memory of the immune system can produce the immunoglobulin without local secretion. In the host-parasite interaction on the intestinal mucosa, many cells or immunoglobulins have been demonstrated for the immune reaction, such as goblet cells, mucosal mast cells, intraepithelial lymphocytes, eosinophils, IgG, IgM, and IgA. Of them, IgA was only observed in *E. caproni* infection (Agger *et al.*, 1993). In the mice infected with six metacercariae of *E. caproni*, worm specific serum IgA increased from 14

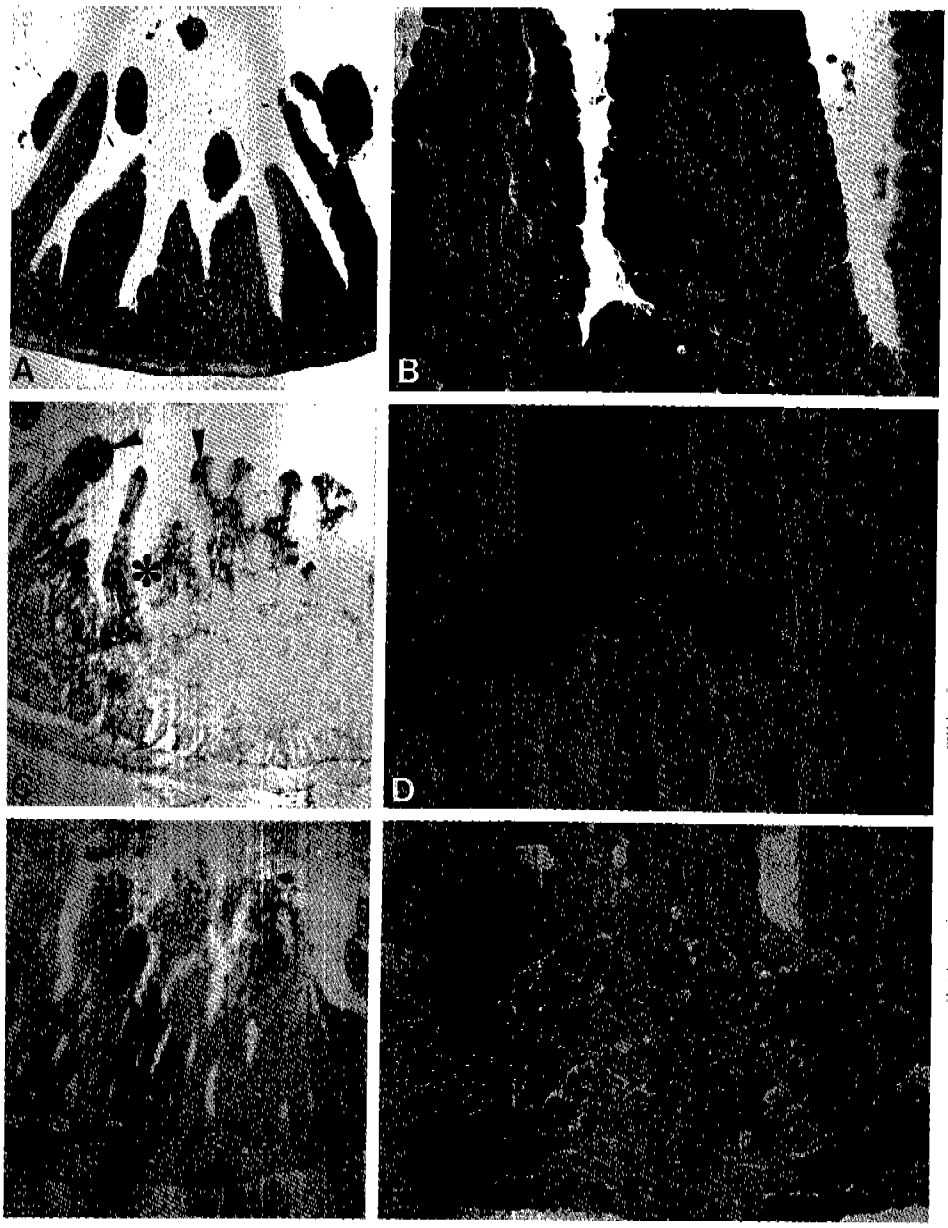


**Fig. 2.** Mouse duodenum stained immnuohistochemically for the non-specific IgA antibody. **Fig. 2A.** Mouse duodenum, control. The villi to crypt ratio was 3:1-4:1. IgA positive reaction (brown color) is shown in the lamina propria and submucosa, X 100. **Fig. 2B.** *ibid.* Asterisk (*) in 2A, X 400. **Fig. 2C.** Mouse duodenum 7 days PI. Villi to crypt ratio is about 1:1. IgA positive reaction was shown more in the lamina propria of the villi than in area between crypts. Arrow heads shows the stained epithelial cells, X 1000. **Fig. 2D.** *ibid.* Asterisk (*) in 2C, X 400. **Fig. 2E.** Mouse intestine 28 days PI. Villi to crypt ratio is 1:1 to 2:1. Weak IgA-positive reaction is shown in the lamina propria and submucosa, X 100. **Fig. 2F.** *ibid.* Asterisk (*) in 2E, X 400.

days PI and reached at peak 28 days PI. When the number of metacercariae was 25, specific IgA increased continuously till 70 days PI. It meant the presence of worm could stimulate the IgA secretion. Our study did not follow-up after 28 day PI. A long term observation could

show the result of immune reaction in chronic infection.

In this study, we could demonstrate the presence of IgA in the mucosal epithelium where the worm attached directly. However, it is another phenomenon from the worm expulsion, because the worm recovery rate did not decrease significantly 4 weeks PI in Balb/C mouse (Kim, 1995). It is speclated that the IgA flows from the lamina propria to epithelium to respond against the worm, since the epithelial cells can not secrete IgA. It is derived from plasma cells. Seven days PI, the amount of IgA in the mucosa increased and it decreased from 28 days PI when worms were in the stage of expulsion. The precise route of flow of IgA is an interesting topic required further studies. It may diffuse through the desmosome between cells, and plasma membrane.

## REFERENCES


Agger MK, Simonsen PE, Vennervald BJ (1993) The antibody response in serum, intestinal wall and intestinal lumen of NMRI mice infected with *Echinostoma caproni*. *J Helminthol* **67:** 169-178.

Heyworth MF (1989) Intestinal IgA response to *Giardia muris* in mice depleted fo helper T lymphocytes and in immunocompetent mice. *J Parasitol* **75:** 246-51

Hong ST (1982) Studies on intestinal trematodes in Korea VII. Growth, development and recovery of *Fibricola seoulensis* from experimentally infected rats and mice. *Korean J Parasitol* **20:** 112-122.

Hong ST, Chai JY, Lee SH (1986) Ten human cases of *Fibricola seoulensis* infection and mixed one with *Stellantchasmus* and *Metagonimus*. *Korean J Parasitol* **24:** 95-97.

Hong ST, Cho TK, Hong SJ, *et al.* (1984) Fifteen human cases of *Fibricola seoulensis* infection in Korea. *Korean J Parasitol* **22:** 61-65.

Hong ST, Shoop WL (1994) *Neodiplostomum seoulensis* N. comb (Trematoda: Neodiplostomidae). *J Parasitol* **80:** 660-663.

Huh S, Lee SU, Huh SC (1994) A follow-up examination of intestinal parasitic infections of the Army soldiers in Whachon-gun, Korea. *Korean J Parasitol* **32:** 61-63.

Kho WG, Chai JY, Chun CW, *et al.* (1990) Mucosal mast cell responses to experimental *Fibricola seoulensis* infection in rats. *Seoul J Med* **31:** 191-199.

Kho WG (1992) Analysis of antigenic proteins of *Fibricola seoulensis*. Ph.D. thesis. Seoul National University.

Kim TK (1995) Mucosal mast cell responses to *Neodiplostoma seoulensis* infections in trhee strains of mice. Ph.D. thesis. Seoul National University.

Lee SH, Yoo BH, Chai JY, *et al.* (1985) A histopathological study on the intestine of mice and rats experimentally infected by *Fibricola seoulensis*. *Korean J Parasitol* **23:** 58-72.

Leyva O, Rico G, Ramos F, Moran P, Melendro EI, Ximenez C (1992) Inhibition of adhesion process mediated by anti-*Entamoeba hitolytica* speicific monoclonal IgA anitbodies. *Arch Med Res* **23:** 227-229.

Nash PV and Speer CA (1988) B-lymphocyte responses in the large intestine and mesentric lymph nodes of mice infected with *Eimeria falciformis* (Apicomplexa). *J Parasitol* **74:** 144-152.

Seo BS, Lee SH, Hong ST, Hong SJ, *et al* (1982) Studies on intestinal trematodes in Korea V. A human case infected by *Fibricola seoulensis* (Trematode: Diplostomatidae). *Korean J Parasitol* **20:** 83-99.

Van Loveren H, Osterhaus ADME, Rangel J, *et al.* (1988) Detection of IgA antibody-producing cells specific for ovalbumin or *Trichinella spiralis* in the rat. *Scan J Immunol* **28:** 377-381

Vanayak VK, Khanna R, Kum K (1991) Kinetics of intraepithelium and lamina propria lymphocyte responsible during Giardia lamblia infection in mice. *Microb Pathog* **10:** 343-50.

Yu JR, Hong ST, Chai JY, Lee SH (1992) The effect of reinfection with *Neodiplostomum seoulensis* on the histopathology and activities of brush border membrane bound enzymes of the rat small intestine. *Korean J Parasitol* **33**(1): 37-43.

=초록=

# 서울주걱흡충 감염 마우스의 IgA 반응

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서울주걱흡충감염 마우스의 혈청내 IgA와 소장의 IgA의 반응을 알아보려고 하였다. 서울주걱흡충의 피낭유충 200마리씩을 Balb/C 마우스에 경구 감염시킨 뒤 3, 7, 14, 28일에 희생시켜, 혈청에서의 서울주걱흡충 특이 IgA를 면역효소법으로 측정하였다. 또한 소장에서의 IgA를 면역조직화학법으로 반응을 관찰하였다. 감염 7일째부터 혈청내 특이 IgA의 역가가 증가하기 시작하여 28일째도 지속되었다. 소장 상피세포에서의 IgA 반응은 감염 14일에 가장 강하였다. 이 결과로 보아 서울주걱흡충 감염 Balb/C 마우스에서 감염후 혈청내 특이 IgA가 증가하고 소장에서 국소반응이 나타나나 충체 배출에는 큰 영향을 미치지 못하는 것으로 생각한다.

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