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 seoulensis*. 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 *Neodiplostomum* 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 immunosorbent 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* entrap 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
(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% pepticin 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 Husbandry 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% H₂O₂. The reaction was stopped in 30 minutes by adding 8 M H₂SO₄. 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 observed in the submucosa and the lamina propria between crypts. The lamina propria of
DISCUSSION

In this study, we checked circulating IgA in sera and secretory 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 intestine to *N. seoulensis* was also seen in the villous epithelial cells. The secretory 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. lambliia* 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 propria (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
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 speculated that the IgA flows from the lamina propria to epithelium to respond against the worm, since the epithelial cells cannot 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


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서울주격흡충 감염 마우스의 IgA 반응

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한림대학교 의과대학 기생충학교실1), 왜부학교실2), 외과학교실3)

서울주격흡충감염 마우스의 혈청내 IgA와 소장의 IgA의 반응을 알아보려고 하였다. 서울주격흡충의 피낭유충 200마리씩을 Balb/C 마우스에 경구 감염시킨 뒤 3, 7, 14, 28일에 흉생시켜, 혈청에서의 서울주격흡충 특이 IgA를 면역호소법으로 측정하였다. 또한 소장에서의 IgA를 면역조직화학법으로 반응을 관찰하였다. 감염 7일째부터 혈청내 특이 IgA의 여가가 증가하기 시작하여 28일까지 지속되었다. 소장 상피세포에서의 IgA 반응은 감염 14일에 가장 강하였다. 이 결과로 보아 서울주격흡충 감염 Balb/C 마우스에서 감염 후 혈청내 특이 IgA가 증가하고 소장에서 국소반응이 나타나 총체 배출에는 큰 영향을 미치지 못하는 것으로 생각한다.

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