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Detection of Serum IgA and IgE Antibodies in Experimental Animals Infected with *Echinostoma hortense*

Yong-Suk Ryang†, Insik Kim, *Kyu-Jae Lee and Ji-Sook Lee

*Department of Medical Technology, College of Health Science, Yonsei University, Kangwon-Do 220-710, *Department of Parasitology, Wonju College of Medicine, Yonsei University, Kangwon-do 220-701, Korea*

Abstract: The change in mean absorbance values for IgA/IgE in rats and mice infected with *Echinostoma hortense* metacercariae was studied from the 2nd week to the 8th week after infection. Serum and intestinal luminal content (ILC) levels of IgA/IgE were measured by enzyme-linked immunosorbent assay (ELISA). The mean absorbance values obtained from IgA in the rats' ILC increased from the 2nd week to the 8th week after infection. The peak value (0.47± 0.01) appeared in the 8th week. The mean absorbance values of IgE in the rats' ILC didn't increase significantly ($p>0.05$). The worm recovery rate decreased at a slower pace after infection. The duration in which the peak value of IgA in rats' ILC appeared was similar to that in which the worm recovery rate declined significantly. Serum levels of IgA/IgE in mice increased gradually from the 2nd week after infection. The peak value (0.45±0.01) of IgA appeared in the 8th week, and that (0.23±0.02) of IgE appeared in the 7th week after infection. The ILC level of IgA in mice continued to increase after infection, and reached its peak in the 8th week. The change in IgA/IgE in the serum and IgA in the ILC of mice was inversely related to worm recovery rate. As a result of this experiment, it is supposed that IgA/IgE may play an important role in the expulsion of *Echinostoma hortense*.

Key Words: *Echinostoma hortense*, ELISA, IgA/IgE, Worm recovery rate, Rat, Mice

INTRODUCTION

Echinostoma hortense was first reported by Asada[4] in 1926 and human infection of this parasite was reported by Tani *et al.*[22] in 1974. In Korea, the number of human cases increased continuously after the first human case reported by Seo *et al.* in 1983[18].

Lee *et al.* (1990)[12] reported that *E. hortense* was found in the intestinal villi of rats from 1 to 3 days after infection and in the internal cavity of the intestine 7 days after infection. Distinct characteristics of the infection include the destruction and shrinkage of intestinal villi by the *E. hortense* suckers and an increase of crypt[10,12,19].

E. hortense infection causes indigestion, a some abdominal pain, diarrhea, chronic absorption disturbance and duodenal ulcers[13,21]. Ahn and Ryang (1986)[2] reported that the eggs began to be discharged on the 11th day (400~500 EPD/worm) and reached the maximum in

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†Corresponding author:
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the 4th week. The EPD began to decrease gradually in the 6th week and then decreased at a faster pace in the 21th week (800 EPD/worm)[2].

IgA antibodies, which are found not only in the intestine but also in saliva, tears and colostrum, play an important role in the immune response and are closely related to the expulsion mechanism of adult worms. The IgA immune responses have been examined in the serum and intestinal luminal content[1,9]. IgE antibodies increased primarily in allergic diseases as well as in parasitic diseases. IgE antibodies show an extreme increase in patients infected with parasites such as *Toxocara canis, Schistosoma japonicum, Wuchereria bancrofti, Schistosoma mansoni* and *Schistosoma haematobium*[6,8,11]. The maximum value of the antibodies and the immune response depend on the kind of parasite, the number of metacercaria and the kind of experimental animal[9]. In general, the cell-mediated immunity of the host is known to be more important than the humoral immunity to the parasites infected in the intestinal cavity. This subject has been investigated in trematodes and nematodes which parasitize in the intestinal cavity. The parasites are suppressed and destroyed by the immune response of the host. Once infected the parasites are discharged spontaneously during the infection period.

In spite of the immunological phenomenon of intestinal trematodes, the mechanism has not yet been completely explained. Thus we studied how the IgA and IgE antibodies in the serum and the ILC affected on *E. hortense* which was used to experimentally infect the rats and mice.

MATERIALS AND METHODS

1. Oral infection of *E. hortense* metacercaria in the experimental animal

We obtained rats (Sprague-Dawley) and mice weighing approximately 150 grams and 30 grams, respectively, at the Korean Experimental Animal Center. The metacercariae of *E. hortense* were separated from *Misgurnus anguillicaudatus* caught in the Somjin-gang (River). The experimental animals were divided into two groups, the control group and experimental group, and each of 8 experimental groups consisted of 5 rats and 5 mice respectively. The animals were orally infected with 150 metacercariae for the rats and 30 metacercariae for mice.

2. Preparation of crude antigen

The adult worms separated from loaches were washed with 0.01 M PBS (phosphate buffered saline) and were homogenized by the homogenizer and ultrasonicator. We collected supernatant as crude antigen after precipitating the specimens by centrifugation at 15,000 rpm at 4℃ for 1 hour. Protein concentration was measured by the Lowry method[14].

3. Collecting of serum and intestinal luminal content

We collected blood from the veins located in the tail and eyeball for 8 weeks. The serum of the control group was collected before the infection. To collect ILC, we cut the intestine of the rat and mice then washed the intestinal cavity with 1 M PBS (pH 7.4). The supernatant was separated from the washed solution from the intestinal cavity after centrifugation.

4. Antibody detection by ELISA

The enzyme-linked immunosorbent assay (ELISA) was performed according to the Voller method[23] for antibody detection. For antigen coating, each plate wells (Nunc, Roskilde, Denmark) was incubated at 37℃ for 1 hour with 100 μl crude antigen diluted with 0.05 M carbonate-bicarbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) to produce a protein concentration of 5 μg/ml. The plate was washed three times with PBST (phosphate buffered saline-0.05% Tween 20) with a 5-minute interval between washings. And each well was incubated with 200 μl 1% BSA (bovine serum albumin)/PBST at 37℃ for 1 hour for block-

Table 1. Chronological changes in the mean absorbance values for IgA and IgE in the serum of the animals infected with *Echinostoma hortense*

Weeks	*IgA		*IgE	
	Rats	Mice	Rats	Mice
**Control	0.06±0.01	0.09±0.01	0.06±0.01	0.06±0.01
2	0.06±0.00	0.14±0.03	0.06±0.01	0.13±0.01
3	0.06±0.00	0.15±0.03	0.05±0.00	0.15±0.02
4	0.06±0.00	0.17±0.02	0.05±0.00	0.16±0.03
5	0.05±0.00	0.23±0.03	0.09±0.01	0.19±0.03
6	0.06±0.00	0.30±0.03	0.05±0.00	0.22±0.02
7	0.07±0.01	0.39±0.04	0.05±0.01	0.23±0.02
8	0.07±0.01	0.45±0.01	0.05±0.01	0.16±0.01

*Mean±SD.
**Control: group of rats and mice subjects 8 weeks after the beginning of the experiment

ing the unattached antigen. After the washings, each well was incubated with 100 μl of serum diluted to 1:100 with PBS-Tween at 37℃ for 1 hour. Additionally, the wells were incubated with 100 μl horseradish-peroxidase conjugated goat anti-mouse IgA (1:10,000 with PBST, Sigma, USA), horseradish-peroxidase conjugated rat anti-mouse IgE (1:1,000 with PBST, Serotec Oxford Kidlinton, United Kingdom) at 37℃ for one hour. For the experimental rats, 100 μl horseradish peroxidase conjugated mouse anti-rat IgE and biotin conjugated mouse anti-rat IgA (1:3,000 with PBST, Serotec) was used. After the final washing, the plates which had been treated with biotin conjugated mouse anti-rat IgA were incubated at 37℃ for 1 hour, then 100 μl of avidin-alkaline phosphatase diluted to 1:1,000 was added. After this final washing, the plates were incubated for 1 hour with 100 μl of a substrate solution (ortho-phenylenediamine 0.5 mg, 30% H_2O_2 10 μl, 0.1 M phosphate citrate (pH 5.0) 50 ml) for HRP and that (1% diethanolamine 10 ml, $MgCl_2$ 1 mg, PNPP 10 mg) for AP. The reaction was stopped by using 50 μl of 2 N H_2SO_4 for HRP and 3 M NaOH for AP. The mean absorbance values were read on the ELISA reader (Molecular Devices) at 490 nm for HRP and 405 nm for AP.

RESULTS

1. Changes in IgA and IgE antibody values in rats

In the serum of the rats, IgA and IgE antibody values didn't increase significantly ($p>0.05$) (Table 1). In the ILC of the rats, the IgA antibody value increased continuously after infection and reached a peak value (0.47±0.11) in the 8th week. The IgE antibody value didn't reveal a statistically significant difference ($p>0.05$) (Fig. 1).

2. Recovery rate of the adult worms in rats

Weekly measurements recorded the recovery rate of adult worms in infected rats to be 28.4%, 24.0%, 24.8% and 23.1% from the 2nd week to the 5th week respectively, showing a decreasing trend. Also, in the 7th and 8th week, this rate showed a significant difference as 12.9% and 5.5%, respectively ($p<0.05$) (Fig. 2).

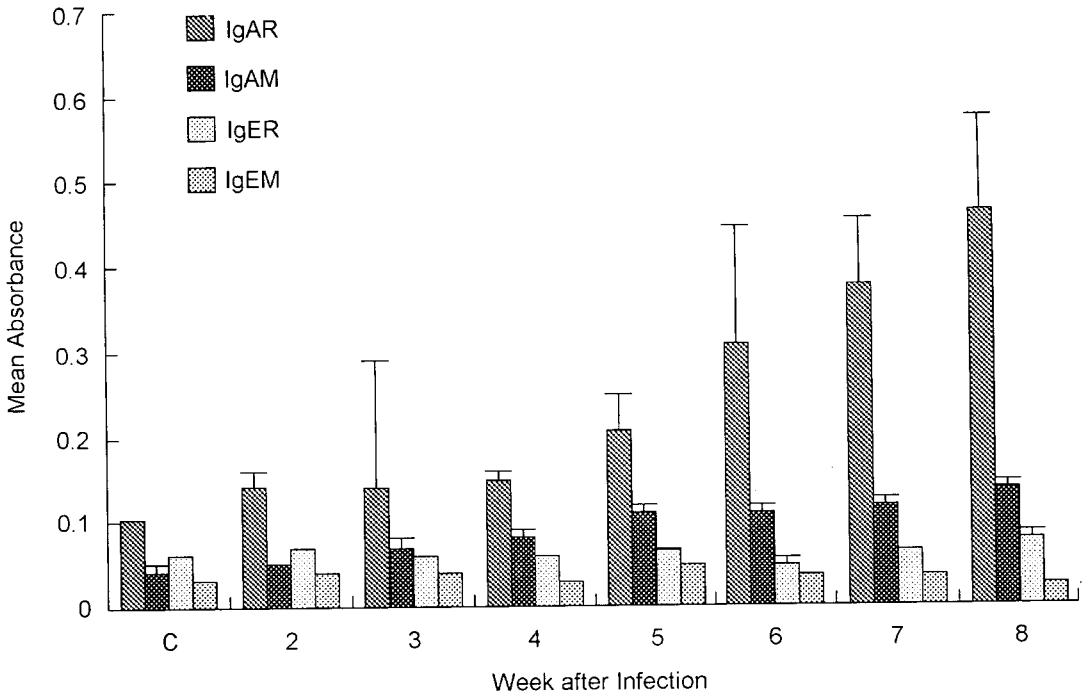

Fig. 1. Chronological changes in the mean absorbance values for IgA and IgE in the intestinal luminal content of the animals infected with *Echinostoma hortense* (R: Rat, M: Mice).

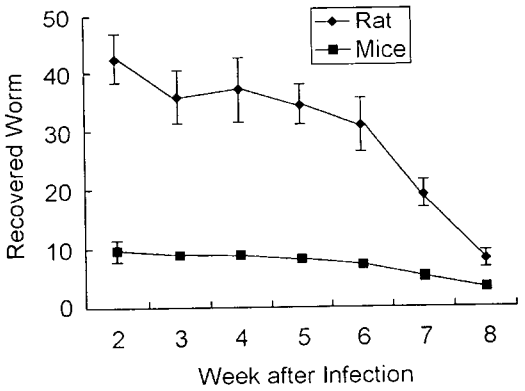

Fig. 2. Chronological changes in the worm recovery rate in the animals infected with *Echinostoma hortense* (Rat: infection with 150 metacercariae, Mice: infection with 30 metacercariae).

3. Changes in IgA and IgE antibody values in mice

In the serum of the infected mice, the mean absorbance value of IgA antibodies showed a statistically significant increase ($p<0.05$) compared to the mean absorbance value of the control group (0.09 ± 0.01) during the experimental periods and showed a maximal absorbance value (0.45 ± 0.01) in the 8th week. In the case of the IgE antibodies, the mean absorbance value significantly increased until the 7th week after infection compared to the control group (0.06 ± 0.01) ($p<0.05$), which showed a maximal absorbance value (0.23 ± 0.02) in the 7th week, then decreased (0.16 ± 0.01) (Table 1). In the ILC, the mean absorbance value of IgA antibodies marked a significant increase ($p<0.01$) compared to the control group (0.04 ± 0.01) from the 3rd week, and their peak value (0.14 ± 0.01) was detected in the 8th week. But the IgE antibodies showed an insignificant difference ($p>0.05$) (Fig. 1).

4. Recovery rate of the adult worms in mice

Weekly measurements recorded the recovery of adult worms in infected mice to be 32.0%,

30.0%, 30.0%, 27.0% and 24.0% from the 2th week to the 6th week respectively, a similar result to that found in the rats. We confirmed this significant expulsion of adult worms through the recovery rate in the 7th week and 8th week (Fig. 2).

DISCUSSION

IgA and IgE antibody levels are dependent on the type of parasite and host under study[15,20]. In intestinal parasites, IgA antibody is secreted from the plasma cells, it then becomes secretary IgA after connecting with glycoprotein in the epithelial cells and being secreted. The IgA is a local antibody and causes immune expulsion of the parasites[16,17]. In the case of mice infected with *Neodiplostomum seoulensis*, IgA antibody values increased continuosly until the 4th week, and in the infected cattle with *Fasciola hepatica*, the IgA antibody level achieved its peak value from the 4th to the 6th week[7,9]. When mice were infected with metacercariae of *Echinostoma caproni*, the IgA antibody value reached its maximum in the 6th week to the 6 metacercaria, but increased continuously until the 10th week to the 25 metacercariae[1]. These results suggest that the phenomena of immune response are revealed differently according to the kind and the number of parasites affecting the host.

IgE is known as a special immune antibody and related to mast cells and eosinophil. That is, IgE antibodies are produced in the plasma cells and are then conjugated with mast cells, which causes the immune response in animals sensitized with an antigen.

In this study, no statistical significance existed in either the IgA or the IgE antibody values of rat serum. Also, IgA antibody values of ILC increased from the 2nd week to the 8th week continuously but did not show statistical significance. Furthermore, the IgE antibody level was low for the duration of the experiment. The recovery rate of adult *E. hortense* worms showed a decreasing trend after infection, and this result indicated that IgA and IgE antibodies are not inolved with the expulsion of adult worms.

In the mouse serum IgA antibody values increased continuously until the 8th week after infection. IgE antibody levels generally increased until the 7th week after infection, then showed a decreasing tendency from the 7th week. In ILC, the IgA antibody value also increased from the 2nd week to the 8th week.

In this study, IgA antibody values in ILC of mice and rats increased continuously until the 8th week after infection, so this resut was similar to the change in serum IgA antibody value. The change in the recovery rate was very similar between rats and mice, suggesting that the IgA in rats and mice played an important role in the local immune defense against the parasite.

This result also coincides with the reports that IgA-containing cells were predominant among the antibody producing lymohocytes in the intestine of mice infected with *Hymenolepis tapeworms*[3,5], and that only IgA antibodies were observed in the 4th week after infection with *Echinostoma caproni*. This figure was especially high at the lower part of intestine[1]. Our research, therefore, supports the importance of IgA antibodies in the local immune defense against parasites[8].

We compared the roles of IgA and IgE antibodies by studying the expulsion and recovering rate of the adult worms in the infected mice and rats. We detected antibodies in the serum and ILC which were related to the local immune response. There was a statistically significant increase in the IgA and IgE antibody values in mouse serum, the IgA antibody value in ILC of mice and the IgA antibody value in ILC of rats. However, all of these detected antibodies were not involved with the expulsion of adult worms. Thus, we think that other kinds of immunoglobulin or immunocytes should be studied in the future.

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=국문초록=

호르텐스극구흡충 감염 흰쥐 및 마우스의 IgA/IgE 항체가 반응 추이

연세대학교 보건과학대학 임상병리학과 및 원주의과대학 기생충학교실*

양용석† · 김인식 · 이규재* · 이지숙

호르텐스극구흡충 피낭유충을 흰쥐 및 마우스에 경구 감염시킨 후 실험동물의 혈청 및 장액에 대한 IgA 및 IgE 항체가 변화 추이를 ELISA방법으로 비교 분석하였다. 감염 흰쥐에서 혈청의 경우 IgA와 IgE 항체가를 대조군과 비교해 볼 때 유의한 차이를 나타내지 않았다. 그러나 장액의 IgA 항체가는 감염 후부터 계속 증가하여 8주에서 최고치 (0.47±0.11)를 나타냈다. 감염 흰쥐에서 충체 회수율은 8주까지 계속 충체 수가 감소하는 경향을 보였는데 특히, 장액의 IgA 항체가 성적이 최고치를 나타낸 기간에 유의한 감소 성적을 나타냈다. 감염 마우스 혈청에서의 IgA 항체가는 감염 기간동안 계속적으로 증가하여 감염 8주에서 최고치 (0.45±0.01)를 보였으며, IgE의 경우는 감염 후부터 7주까지 증가하여 7주에서 그 최고치 (0.23±0.02)를 보였으나 그 이후 다시 감소되었다. 장액의 경우 IgA 항체가는 감염 후부터 증가하여 8주에서 최고치 (0.14±0.01)를 보였으며, 이는 혈청 IgA의 항체가 변화와 유사한 경향을 보였다. 감염 마우스의 충체 회수율은 감염 후부터 계속적으로 감소하였으며 7주와 8주에서 유의한 감소를 보였는데 ($p<0.05$) 이것은 장액에서의 IgA와 IgE, 혈청에서의 IgA의 항체가 최고치를 나타낸 기간과 일치하였다. 이상의 결과에서 호르텐스극구흡충 감염 마우스에 있어서, IgA 및 IgE 항체가의 유무는 충체의 배출 (expulsion)에 일부 연관되어 있음을 알 수 있었다.

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†별책 요청 저자