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An Immunopathological Study of the Liver of the Mice Infected with *Toxocara canis*

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Introduction

Observations on the histological responses elicited by *Toxocara canis* have been made in some animals (Done *et al.*, 1960; Schaeffer, 1960; Beaver, 1962; Galvin, 1964; Fernando, 1968; Aljeboori *et al.*, 1970). In particular, mouse is considered as an useful animal model for histopathological study (Hoeppli *et al.*, 1949; Higashikawa, 1961; Kunishige, 1964; Burren, 1968; Zyngier, 1974; Kayes and Oaks, 1978). These studies have revealed eosinophil-rich granuloma formation surrounding the second stage larvae was the most characteristic finding in *Toxocara* infection. In a single inoculation of *T. canis* eggs, the eggs hatch in the gastro-intestinal tract and the second stage larvae migrate via the portal vein into the liver. Thereafter, most of them migrate into lung. Ultimately, larvae enter the general circulation and migrate into skeletal muscle and central nervous system (Oshima, 1961; Olson, 1962; Kondo *et al.*, 1970); then, the larvae can remain in the sites for a long time (Sprent, 1953).

On the other hand, acquired immunity in mice to *Toxocara* infection has also been demonstrated. It has been shown that previous or multiple infection of mouse with *T. canis* eggs increased in number of larvae recovered from the liver (Lee, 1960; Kato, 1973; Kondo *et al.*, 1976; Sugane and Oshima, 1983). However, little information has been known on the mechanisms of host immune resistance. Previous studies in our laboratory (Akao, 1985) have suggested that humoral immune responses against some components of excretory-secretory products of *T. canis* larvae played a role in the induction of trapping of the larvae in the liver of reinfected mice. However, no details of immunopathological changes during the course of *Toxocara* infection in mice were obtained.

The present study deals with the detection of the larval antigen reacting with infected rabbit serum and mouse immunoglobulin G around the larvae in the liver of reinfected mice, comparing with the result of primary infection in mice.

Materials and Methods

1. Experimental infection

A total of 38 female BALB/c mice, 35-day-old, were used. Thirteen animals were inocu-

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lated with 500 embryonated eggs of *T. canis* and sacrificed at 12 hr, 1, 2, 3, 7, 28 and 98 days after infection. Twenty mice were inoculated again with the same dose 7 weeks after the first infection and sacrificed at 1, 2, 7 and 14 weeks after reinfection. Five uninfected control mice were sacrificed at the end of the experiment. After autopsy, the liver of each mouse was examined as described below.

2. Immunohistochemical procedures

The tissue slices (2 mm thick) of the liver of the mice were obtained immediately after killing, and were frozen at −40°C, and cut in a cryostat. The specimens were fixed in acetone for 10 min.

Peroxidase anti-peroxidase (PAP) method described by Sternberger *et al.* (1970) was employed for the detection of larval antigens of *T. canis* and IgG deposit in the liver granulomata in mouse infected with *T. canis* eggs. Sections were incubated at 37°C for 1 hr with goat serum (10% in phosphate buffered saline, PBS, pH 7.2) and then left overnight with the serum from chronically infected rabbit 26 weeks after infection (Kondo *et al.*, 1984) diluted 1:1000 in PBS or rabbit anti-mouse IgG (heavy chain specific, Cappel, NY, USA) diluted 1:500 in PBS. After repeated washing, sections were incubated for 30 min with goat anti-rabbit IgG in antibody excess (1:100 in PBS), washed, and finally incubated for 30 min with a 1:100 dilution in PBS of soluble PAP rabbit immune complex (DAKOPATTS, Denmark) after final washing. All sections were preincubated for 40 min in methanol supplemented with H_2O_2 to inhibit endogenous peroxidase activity. Peroxidase activity was revealed by a 5 min incubation in 3-3′, diaminobenzine-4-HCl (0.2 mg/ml in Tris HCl buffer, pH 7.6) supplemented with H_2O_2.

Results

Table 1 summarized the immunohistological changes of the liver during the course of infection. In primarily infected mice from 12 hr to 28 days after infection, the larvae passed through the liver with minimal and transient infiltration of eosinophils surrounding them or perivascular regions. However, no pathological changes were observed by 98 days of infection. Immunohistological observation revealed that the larvae in the liver on day 2 of infection were clearly stained by PAP method using infected rabbit serum as a primary antibody. The site with the most intensive reaction in the larva was excretory cells (Fig. 1). Neither antigen nor IgG was detected around the

Table 1 Immunohistological findings in the liver of mice receiving primary or secondary infection with 500 embryonated eggs of *Toxocara canis*

	Mice with the primary infection (days)									Mice with the reinfection (days)				
	Control	0.5	1	2	3	5	7	14	28	98	7	14	35	49	98
Larvae in liver	−[a]	+[c]	+	−	−	−	−	−	−	−	+	+	+	+	+
Antigen around larvae	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
IgG around larvae	−	−	−	−	−	−	−	−	−	−	±[b]	±~+	+	+	+
Granuloma formation	−	−	−	−	−	−	−	−	−	−	−	±~+	+	+	+
Antigen in granuloma	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
IgG around granuloma	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
IgG in granuloma	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
Eosinophil around larvae	−	−	+	+	+	+	−	−	−	−	++[d]	++	+	+	+
Lymphocyte around larvae	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Plasma cell around larvae	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+

a) negative; b) weak positive; c) moderate positive; d) strong positive.

larvae. Granulomatous lesion in the liver was not found in the mice with primary infection.

Immunohistologically, the liver of reinfected mice was quite different from that of primarily infected mice in respect of an increase of the larvae in the liver. Although the parasites were surrounded by abundant eosinophils 1 week after reinfection, no granuloma formation was seen. Antigens were also demonstrated in the surrounding area. IgG was demonstrated around infiltrated eosinophils even in the location far from the larvae (Figs. 2B, 2C).

Granuloma formation in the liver of reinfected mice appeared after 2 weeks of second infection. The granuloma consisted of abundant eosinophils, epithelioid cells and a few plasma cells. Macrophage-like antigen-containing cells were constantly seen at the central area of the granuloma. Fibrosis of the granuloma developed gradually in the course of reinfection, taking place of eosinophil infiltration.

By the 7th week of reinfection, almost all the larvae in the liver were well encapsulated with thick connective tissues. At this time, infiltration of the eosinophils was subsided comparing those of the liver of mice 2 weeks after reinfection. Amorphous debris were often seen at the central area of the granuloma (Fig. 3A). By PAP staining, antigen and IgG were clearly detected in the debris. Antigen was also strongly stained in the parasite and its vicinity. On the contrary, stained IgG was not found in the parasites, but slightly found around the parasites (Figs. 3B, 3C). Uninfected rabbit serum failed completely to stain the larvae in the granuloma (Fig. 2A), and we could not detect any non-specific staining in the liver of uninfected mouse reacting with the serum from infected rabbit or with anti-mouse IgG serum.

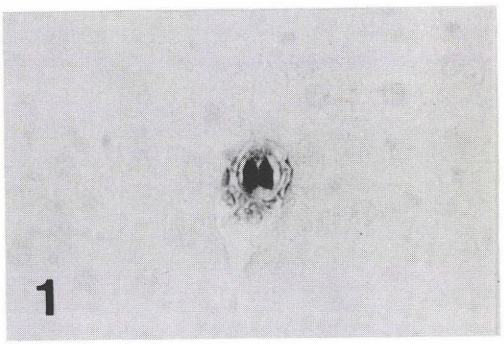

Fig. 1 Cryostat section of the liver of mice reacted with infected rabbit serum 2 days after primary infection (PAP staining, ×400). Staining was concentrated in the excretory cells.

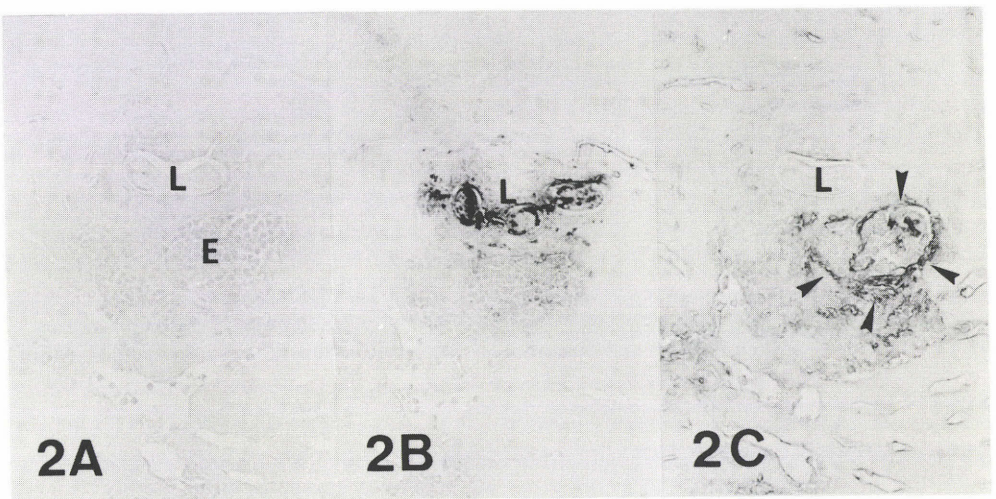

Fig. 2 Cryostat section of the liver of mice 1 week after secondary infection, which was carried out at 7th week of primary infeciton (×400). (A) The larva (L) and eosinophils (E) were not stained when the section was incubated with normal mouse serum. (B) Antigens were demonstrated in or near the larve when the section was incubated with infected rabbit serum. (C) IgG (arrow heads) was seen at a distance from the larva when the section was incubated with goat anti-mouse IgG serum.

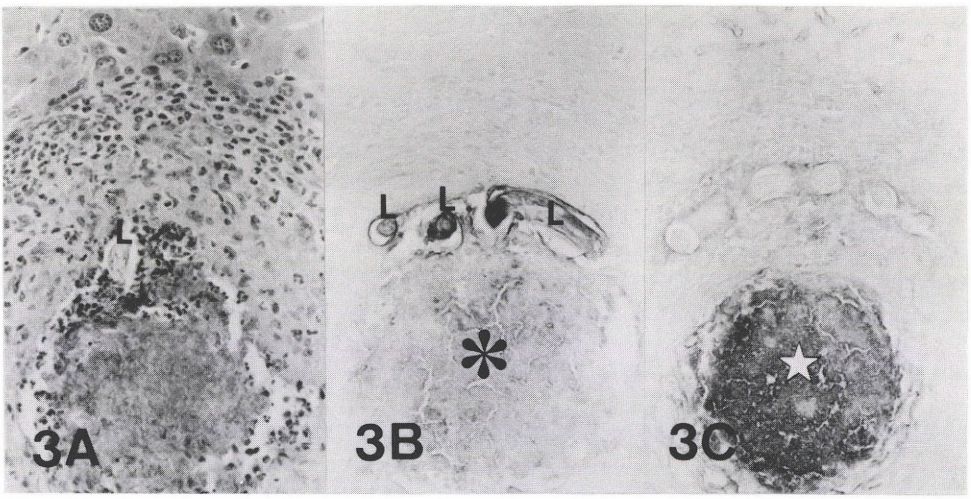

Fig. 3 Cryostat section of the liver of mice 7 weeks after secondary infection (×400). (A) Well developed granuloma containing a larva (L) when the section was stained with haematoxylin-eosin. (B) Antigens were demonstrated in the larva (L) and at the central area of a granuloma (*) when the section was incubated with infected rabbit serum. (C) IgG was observed at the central area of a granuloma (☆) as dark stained mass when the section was incubated with goat anti-mouse IgG serum.

Discussion

In murine toxocariasis, it has been demonstrated that the almost all of the second stage larvae were trapped in the liver of mice receiving reinfection (Lee, 1960; Olson, 1962; Kondo *et al.*, 1976; Sugane and Oshima, 1983). In the previous report, we demonstrated that this larval trapping phenomenon in the liver could be induced by passive transfer of the antibody produced against the larval ES products of *T. canis* (Akao, 1985). The present study clearly demonstrated that antigen derived from the second stage larvae of *T. canis* and host immunoglobulin G coexisted in the hepatic granuloma in reinfected mice. Though the antigen was observed around the larvae until granulomatous lesions were well developed, IgG could not be detected in the same area but around eosinophils which were even in the location far from the parasite. These findings suggested that the larvae might actively migrate in the liver, and continuously produce the ES antigen. Thus, the antigen will stimulate both the proliferation of eosinophils and the production of host IgG.

It is not clear how the larval ES antigen plays a role in granulomatous inflammation of toxocariasis. In experimental ocular toxocariasis in mouse, Ghafoor *et al.* (1984) suggested that the inflammatory phenomenon in the retina may be propagated by secreted surface antigen of the larvae. Sugane and Oshima (1982, 1983) reported that the phenomenon of the larval trapping in the liver of reinfected mice was a T cell dependent response, and the larvae might be killed in eosinophilic granulomas. Furthermore, they demonstrated that the larval ES products could induce eosinophilia (Sugane and Oshima, 1984). We observed that passive transfer of the serum from reinfected mice could induce the larval trapping in the liver of recipient nude mice following challenge infection (unpublished data). We therefore speculate that humoral immunity and eosinophils, which are induced as a result of secondary immune response to reinfection, probably reflect the larval trapping in the liver of mice receiving reinfection.

It is of interest to note that antigen-containing cells were seen at the central area of the

granuloma. Kayes and Oaks (1978) observed by an electron microscope that a central cellular region of macrophages and epithelioid cells of the muscle granuloma contained striking aggregations of glycogen at 14 days of infection. Further studies should be needed to explore whether the aggregated glycogen in the macrophages are identical with the antigenic substances which we observed in the liver granulomas of mice receiving reinfection.

Summary

Immunohistological investigations were carried out to examine the antigens and IgG deposit in the granulomatous inflammation of the murine toxocariasis. In primarily infected mice, antigen that reacted with the serum from infected rabbit was clearly seen in excretory cells of the larvae. However, neither antigens nor IgG were detected surrounding the larvae.

In reinfected mice, a large number of the larvae were observed in the liver throughout the experiment. Infiltration of abundant eosinophils was seen around the larvae 1 week after reinfection, and IgG was found around them. The antigens were detected not only in the larvae but also around the larvae. Granuloma formations were first found 2 weeks after reinfection. At this time, macrophase-like cells that phagocytosed the antigen were observed in the central area of the granuloma. While degerated macrophages in the granuloma became amorphous debris at 7th week of the reinfection, the antigens and IgG were stained coincidentally in the debris by PAP method.

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犬蛔虫感染マウス肝臓の免疫病理学的研究

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マウスの実験的犬蛔虫症における肝臓の肉芽腫病変を免疫組織学的に検索した．1回感染マウスでは，感染ウサギ血清と反応する幼虫抗原は排泄細胞に強くみられた．しかし，幼虫周囲には抗原もIgGも認められなかった．

重複感染のマウスでは肝臓に多くの幼虫がみられた．再感染後1週目の肝臓の幼虫周囲には多数の好酸球が浸潤し，IgGはこれら好酸球の近傍に観察された．抗原は幼虫内だけではなく，その周囲の組織内にも認められた．肉芽腫は再感染後2週目からみられ始め，抗原を貪食したマクロファージ様の細胞が肉芽腫の中心に存在していた．再感染後7週目になると，肉芽腫内のマクロファージは変性し，無構造物質になったが，この中にはPAP法によって抗原とIgGが共に染色された．