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Research Note

Isolation and Antigenicity of Concanavalin A Binding Outer Surface Glycoconjugates of *Toxocara canis* Larvae

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A considerable interest is currently taken in identification of antigens on surface of helminth such as schistosomula of *Schistosoma mansoni* (Bickel and Ford, 1982; Aronstein *et al.*, 1983) or *Trichinella spiralis* (Mackenzie *et al.*, 1981) that are the target of protective immune response. Concerning protective immune response to *Toxocara canis*, the phenomenon of larval trapping in the liver has been demonstrated by some authors (Lee, 1960; Olson, 1962; Fernando, 1968; Kondo *et al.*, 1976; Sugane and Oshima, 1983). However, it is yet unknown how outer surface of *T. canis* larvae may play a role in the protective immune response during the course of infection.

Present paper dealt with attempts to isolate the larval surface glycoprotein of *T. canis* reacting with biotinylated concanavalin A (ConA), and to collect the surface molecules obtained by avidin D conjugate agarose affinity chromatography, and also to demonstrate the antigenicity of the ConA binding outer surface glycoproteins (ConA-LOS) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by hydrophobic transfer of the polypeptides to nitrocellulose membrane (Southern blotting technique).

Second stage larvae and larval ES antigen of *T. canis* were prepared from adult female *T. canis* according to the method of Koizumi *et al.* (1983). After centrifugation at 2,000 rpm for 5 min, the pellet of larvae (1.1×10^6 larvae) was suspended in cold (4°C) phosphate buffered saline (pH 7.2, PBS). Then the larvae were washed extensively in cold PBS to remove foreign materials and allowed to settle before incubation with biotinylated Con-A (Vector Lab. Inc. Calif., USA, 5 μg/ml) at 4°C for 30 min. The supernatant fluid was then collected by centrifugation at 2,000 rpm for 5 min at 4°C. These procedure were repeated 5 times. The pooled supernatant fluid was centrifugated at 30,000 g for 1 hr at 4°C and was dialysed against distilled water, then the final supernatant fluid was concentrated by the pressured dialysis using cellulose tube to 2 ml. The incubation products contained ConA binding glycoconjugates on larval outer surface were submitted to affinity chromatography on immobilized avidinD: Avidin D agarose (Vector Lab. Inc., USA) Avidin D agarose was thoroughly washed in PBS, pH 7.2. To a column containing 3 ml of gel were added 2 ml of incubation products dissolved in the elution buffer. Nonretained material was eluted with the buffer at room temperture. Retained material was eluted with 0.2 M metyl-α-D-mannopyranoside (P-L Biochemicals, Inc., Milwaukee, USA) in the elution buffer. This fraction was dialysed against distilled water prior to lyophilization, then used for SDS-PAGE analysis. SDS-PAGE and immunoblotting were performed as previously described

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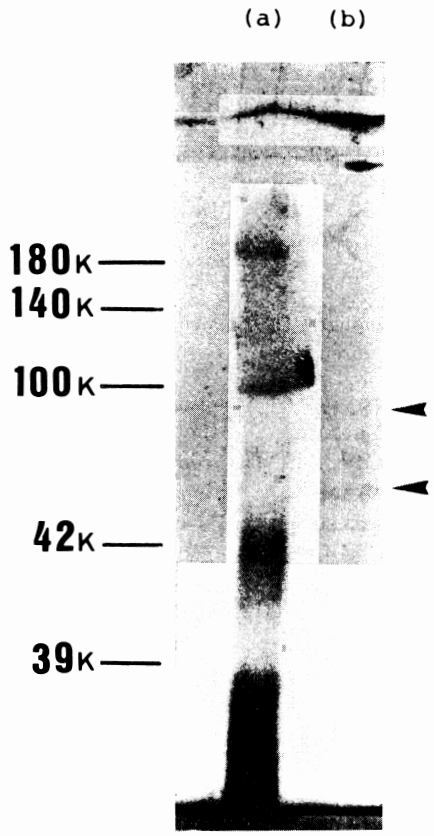

Fig. 1 SDS-PAGE analysis of larval excretory-secretory products (a) and concanavalin A binding larval outer surface glycoproteins (b) of *Toxocara canis* followed by silver staining. The position of molecular weight markers is indicated on the left side. Arrows indicate concanavalin A binding outer surface glycoprotein bands with molecualar weight of 100,000 and 80,000.

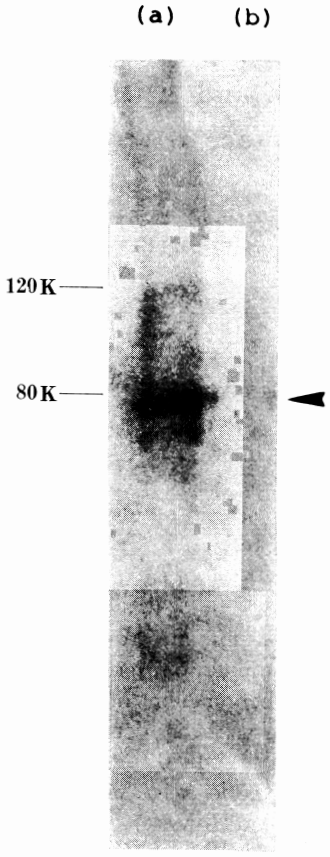

Fig. 2 Antigenicity of larval excretory-secretory products (a) and concanavalin A binding larval outer surface glycoproteins (b), when (a) and (b) reacred with infected rabbit serum taken from 26 weeks after infection of 1×10^5 embryonated eggs of *Toxocara canis*. Arrow indicates 80,000-molecualr-weight antigenic glycoprotein.

(Akao *et al.*, 1983) and immune sera were raised in rabbit infected with 1×10^5 embryonated eggs of *T. canis* 2 or 26 weeks after infection. Proteins with molecular weights ranging from 39,000 to 180,000 (SDS-PAGE Marker I, Seikagaku Kogyo Co., Ltd., Tokyo, Japan) were used as markers.

By using the procedure described above, 1.8 mg dry-weight of ConA-LOS were recovered from 1.1×10^6 larvae. Namely, about 0.3 ng of ConA-LOS on each larva were shedded into culture medium for 30 min. Silver stain of gel after SDS-PAGE revealed at least 2 components with molecular weight of 80,000 and 100,000 in ConA-LOS (Fig. 1). Upon reaction of the ConA-LOS with infected rabbit sera using immunoblotting technique, the 80,000-molecular-weight glycoprotein reacted with serum taken from rabbit 26 weeks after infection (Fig. 2), but did not react with serum from rabbit 2 weeks after infection. In contrast, the 100,000-molecular-weight glycoprotein on the larval surface could not be recognized by antibodies from infected rabbit.

The results obtained here suggested that

T. canis larvae had glycoconjugates, which consisted of at least two glycoprotein componenst, on their outer surface binding with ConA and one (MW : 80,000) of them had antigenicity. Recently, Maizeles *et al.* (1984) reported that a set of molecules consisted of molecular weight 32,000 and 120,000 in the surface antigens of *T. canis* larvae were antigenic to infected host. The difference between their results and ours might be ascribed to the difference of strategy of the experiments. They analysed the antigens by radioimmunoprecipitation reacted with human and mouse sera, while we used immunoblotting technique to detect the antigenicity of ConA-LOS reacting with rabbit serum. Further studies for understanding of the interrelation between the immune recognition against larval outer surface antigens and the course of infection with *T. canis* should be needed.

Acknowledgment

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Reference

1) Akao, N., Kondo, K., Okamoto, T. and Yoshimura, H., (1983): Antigenic analysis of excretory-secretory products of second stage larvae of *Toxocara canis* and the antigen recognition in the course of infection. Jpn. J. Parasitol., 32, 7-14 (in Japanese).
2) Aronstein, W. S., Norden, A. M. and Strand, M. (1983): Tegmental expression in larval and adult stages of a major schistosome structural glycoprotein. Am. J. Trop. Med. Hyg., 32, 334-342.
3) Bickel, Q. D. and Ford, M. J. (1982): Studies on the surface antigenicity and susceptibility to antibody-dependent killing of developing schistosomula using sera from chronically infected mice and mice vaccinated with cercariae. J. Immunol., 128, 2101-2106.
4) Fernando, S. T. (1968): Immunological respones of rabbits to *Toxocara canis* infection. Parasitol., 58, 91-103.
5) Koizumi, T. Hayakawa, J, and Kondo, K. (1983): *Toxocara canis*: Immunogenic source of *Toxocara canis* in infected rats. Jpn. J. Parasitol., 32, 359-386.
6) Kondo, K., Shimada, Y., Kurimoto, H. and Oda, K. (1976): Expreimental studies on "visceral larva migrans" 2. On resistance of mice receiving sensitization and challenge with *Toxocara canis*. Jpn. J. Parasitol., 25, 371-376 (in Japanese).
7) Lee, H. F, (1960): Effects of superinfection on the behavior of *Toxocara canis* larvae in mice. J. Parasitol., 46, 533-538.
8) Mackenzie, C. D., Jungery, M., Taylor, P. M. and Ogilvie, B. M. (1981): The *in vitro* interaction of eosinophils, macrophages and mast cells with nematode surface in the presence of complement or antibodies. J. Pathol., 133, 161-175.
9) Maizeles, R. M., de Savigney, D. and Ogilvie, B. M. (1984): Characterization of surface and excretory-secretory antigens of *Toxocara canis* infective larvae. Parasite Immunol., 6, 23-37.
10) Olson, L. T. (1962): Organ distibution of *Toxocara canis* larvae in normal mice and in mice previously infected with *Toxocara*, *Ascaris* and *Trichinella*. Tex. Rep. Biol. Med., 20, 651-657.
11) Smith, H. V., Quinn, R. and Girdwood, R. W. A. (1981): The effect of temperature and antimetabolites on antibody binding to the outer surface of second stage *Toxocara canis* larvae. Mol. Biochem. Parasitol., 4, 183-193.
12) Sugane, K. and Oshima, T. (1983): Trapping of large numbers of larvae in the livers of *Toxocara canis*-reinfected mice. J. Helminthol., 57, 95-99.

短　報

Concanavalin A 結合性犬蛔虫幼虫 outer surface 複合糖質の分離とその抗原性

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犬蛔虫第2期幼虫の outer surface に存在する複合糖質のうち，concanavalin A と結合する物質を concanavalin A結合biotinを用いて回収し，avidin結合agarose affinity chromatographyによつて分離した．抗原性についてはSDS-PAGE と Immunoblotting 法を組合せた間接酵素抗体法によつて検討した．その結果，1隻の幼虫は30分間に約0.3ngの複合糖質を分泌し，またこの中には少なくとも2種類（MW：100Kと80K）の蛋白質が含まれていた．このうちMW：80K の画分には抗原性が認められたが，MW：100K 画分は抗原性を保持していなかつた．