[Jpn. J. Parasitol., Vol. 38, No. 6, 361—367, December, 1989]

A Further Study on Specific Antibody Response to Circulating Anodic Antigen in Experimental *Schistosoma japonicum* and *S. mansoni* Infection by Enzyme-Linked Immunosorbent Assay

MIZUKI HIRATA, MASATO TAKUSHIMA AND HIROSHI TSUTSUMI

(Accepted for publication; October 17, 1989)

Abstract

Specific antibody response to circulating anodic antigen (CAA) was studied employing a highly purified antigen in the enzyme-linked immunosorbent assay. In *Schistosoma japonicum*-infected mice, unisexual infection showed greater antibody reaction than bisexual infection. When the relationship between antibody level and worm burden was investigated in bisexual infection, a negative correlation was found ($r=-0.51$, $p<0.05$). In sera of rabbits with 50 cercarial infection, a kinetic study showed that antibodies appeared during a restricted period (4—7 weeks after infection), a time before worms attain fully growth. These findings indicate that the level of anti-CAA antibodies is closely related to the intensity of the infection. When sera from *S. mansoni*-infected mice were tested with *S. japonicum*-derived CAA, they had high levels of antibodies. In addition, the antibody levels were unrelated to the worm burden, differing from *S. japonicum* infection.

Key words: *Schistosoma japonicum,* circulating anodic antigen, antibody, mouse, rabbit, *Schistosoma mansoni*

Introduction

Circulating anodic antigen (CAA) is a major molecule detectable in the sera of animals and humans with schistosome infection (Berggren and Weller, 1967; Deelder *et al.*, 1976; Qian and Deelder, 1983a) and has been characterized as a proteoglycan originating from the cell lining of schistosome gut (Nash, 1974; Nash *et al.*, 1974; Lichtenberg *et al.*, 1974; Nash *et al.*, 1977; Fujino *et al.*, 1985; de Water *et al.*, 1986). Since demonstration of schistosome products would indicate an active infection, and the antigen level might correlate with the worm burden, much effort has been devoted to the exploration of CAA. A recent study has shown that CAA can be detected at a level of less than 1ng/ml human serum, when a monoclonal antibody was applied in a sandwich enzyme-linked immunosorbent assay (ELISA) (Deelder *et al.*, 1989). This strongly suggests the practical usefulness of CAA detection for the epidemiological study of schistosomiasis.

There have been several reports which describe frequent occurrence of anti-CAA antibodies in *Schistosoma mansoni*-infected animals or man (Deelder, 1973; Deelder *et al.*, 1978, Nash, 1978; Nash *et al.*, 1978), suggesting that antibody detection might be useful in the diagnosis of schistosomiasis. Studies on *S. japonicum* infection are, however, incomplete (Qian and Deelder, 1983b; Qian and Wen, 1983; Hirata *et al.*, 1988). Although CAA has been reported to be genus specific (Nash *et al.*, 1974), accurate comparison of molecular constituents has not been performed, and biological features differ with each species. Therefore, immunological response to CAA must be studied separately.

In a previous study with counter immunoelectrophoresis (CIE), we reported that the incidence of anti-CAA antibodies inversely correlated with the worm burden in *S. japonicum*-infected mice (Hirata *et al.*, 1988). The present study was performed to more accurately deter-

Department of Parasitology, Kurume University School of Medicine, Kurume 830, Japan
平田瑞城　多久島匡登　塘　普（久留米大学医学部寄生虫学講座）

mine the relationship with the ELISA test, using highly purified CAA as the antigen.

Materials and Methods

Animals and infection

Female 6-week-old ddY mice and rabbits weighing 2.0Kg were used.

In the previous study (Hirata, *et al.*, 1988), a part of mice received 20–30 *S. japonicum* (Japanese strain) cercarial injection were found to be unisexual infection. These mice were used in this study for comparison of ELISA and CIE test. Groups, consisting of 2–6 mice each, had the infection periods of 4, 6, 10, and 20 weeks. Bisexually infected mice with *S. japonicum* or *S. mansoni* were prepared by exposing subcutaneously to approximately 30 cercariae. Groups consisting of 5–8 mice for each infection were necropsied at 14, 27 and 44 weeks after infection. *S. mansoni*-infected snails, *Biomphalaria grabrata* (Puerto Rican strain), were generously supplied by the Department of Medical Zoology, Faculty of Medicine, Kagoshima University. Blood samples were taken by cardiac puncture, and sera were stored at −70°C until use. The worms were recovered by the perfusion method and counted.

S. japonicum-infected rabbits were investigated in 2 experiments. Two rabbits infected with 50 cercariae were bled before infection and then weekly or biweekly, up to 14 weeks. Six rabbits infected with 500 cercariae were treated with praziquantel between 23 to 36 weeks after infection. The drug was administered orally at a daily dose of 300mg per rabbit for 4 consecutive days. Blood and stool samples were taken before and after treatment. Egg excretion was examined by the ethylether formalin concentration technique.

Antigen preparation

Crude CAA extracts were obtained by phenol treatment of *S. japonicum* adult worm homogenates, as previously described (Hirata, 1981), and further purified, essentially according to the method of Nash *et al.* (1977; 1981). After dialysis against 0.05M phosphate buffered saline, pH 7.2 (PBS), the extract was applied on a DEAE-cellulose column. The column was extensively washed with PBS and the adhering fractions were eluted with 1M NaCl in PBS. Fractions retained in the column were further eluted with a linear gradient of 1M NaCl + 0.2M HCl. A CAA active peak coinciding with the HCl concentration of 0.05M was collected (Fig. 1). The fraction was dialyzed against distilled water, lyophilized, and weighed. The specificity was analysed in the subsequent ELISA test.

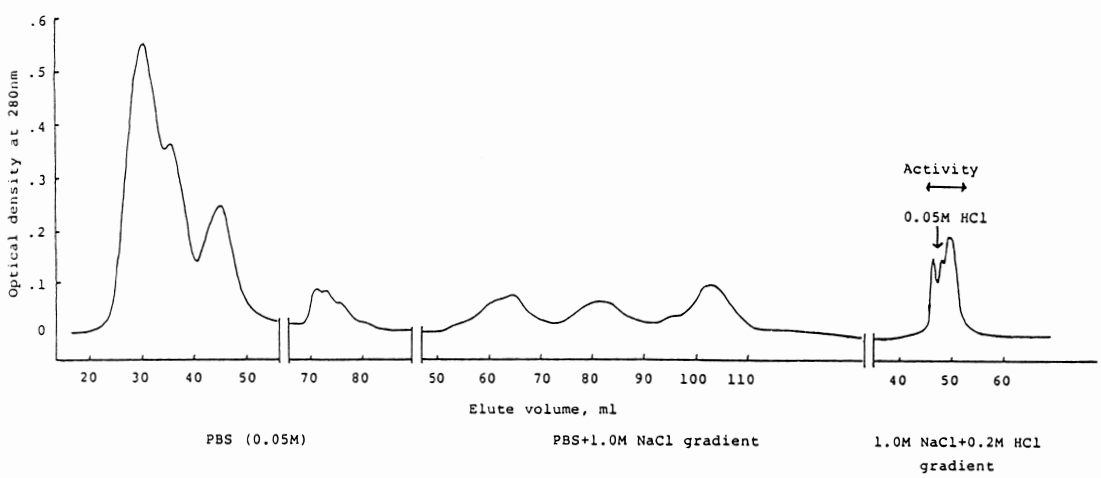

Fig. 1 Elution pattern of *S. japonicum* adult worm phenol extract from DEAE cellulose column. A fraction eluted at approximately 0.05M HCl concentration showed CAA activity on counter immunoelectrophoresis.

Enzyme-linked immunosorbent assay (ELISA)

The method of Kelsoe and Weller (1978) was used, with a slight modification. Briefly, plates (Dynatech, M129A) were precoated with 100 μl of poly-1-lysin (100 μg/ml PBS), and were then sensitized with 100 μl of CAA (2 μg/ml PBS). Non-specific reaction was blocked with 5% bovine serum albumin (BSA). Serum dilution was used at 1:20. Peroxidase-conjugated goat anti-mouse or anti-rabbit γ chain specific IgG (1:1,000) (Cappel Laboratories) and o-phenylene-diamine-H_2O_2 were used in the subsequent reactions. The optical density (OD) was read at 492nm using the ELISA autoreader (Hitachi-Corona, Co., Ltd). The antibody level was expressed by the OD of antigen-coated well minus the OD background value of the serum. Normal mouse sera (10) and rabbit sera (15) were used as negative controls. In order to examine the specificity of the ELISA test, hyperimmune sera against phenol extract from *S. japonicum* adult worm homogenate, *S. japonicum* egg antigen or adult worm, *Paragonimus westermani,* and *Gnathostoma doloresi,* which had been previously produced using Freund's complete adjuvant in rabbits, were used. All sera were tested in duplicate, and ELISA values were averaged. Values higher than 0.1 were regarded as positive, judging from the upper value of the 95% confidence limit of normal sera.

Results

The specificity of purified antigen was first examined using hyperimmune sera. When antisera against phenol extract from *S. japonicum* adult worms, or adult worm homogenates were used as positive controls, the ELISA values were approximately 1.6 and 0.7, respectively. In contrast, antisera against *S. japonicum* egg antigen, *P. westermani,* and *G. doloresi* showed consistently less than 0.1, while these showed marked reactions with the homologous antigens.

In the previous CIE reaction, sera from mice unisexually infected with *S. japonicum* showed a high incidence of anti-CAA antibody (54.5%) (Hirata *et al.*, 1988). When these mouse sera were tested (Fig. 2), 13 out of 17 sera (76.5%) showed positive reactions, suggesting a higher sensitivity to the ELISA test. Antibodies were detected from the 4th week of infection, and the reactivity appeared to continue until the 20th week, although there was some decrease at the 6th week. The ELISA value of this mouse group was

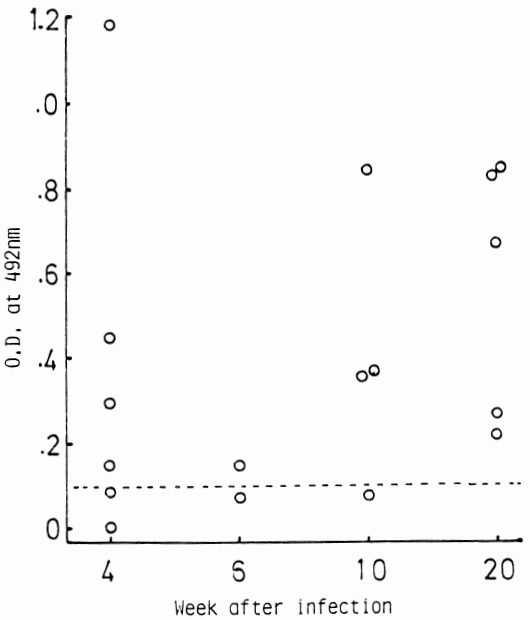

Fig. 2 Levels of anti-CAA antibody in sera from mice with unisexual infection of *S. japonicum*. The animals had only male worms and/or immature worms. ELISA values higher than 0.1 (broken line) were regarded as positive.

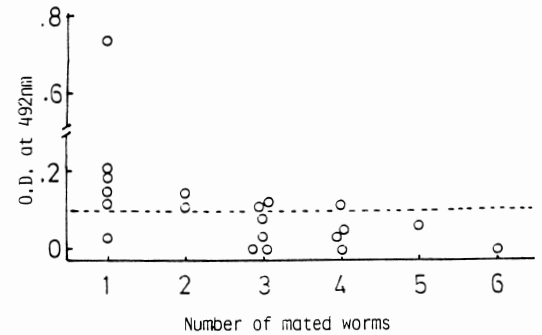

Fig. 3 Levels of anti-CAA antibody in sera from mice with bisexual infection of *S. japonicum*. The animals were necropsied at 14, 27, and 44 weeks after infection. ELISA values higher than 0.1 (broken line) were regarded as positive.

Mean 0.404±SE 0.084.

In bisexual infection of *S. japonicum* (Fig. 3), the summarized positive rate and ELISA value were 50.0% and Mean 0.112±SE 0.035, respectively, apparently lower than those in unisexual infection. When the reactivity was investigated in relation to the worm burden, represented as worm pair numbers, there was a negative correlation ($r=-0.51$, $p<0.05$). The period of infection was unrelated to the serum reactivity.

In rabbits that received 50 cercarial injections (Fig. 4), the appearance of anti-CAA antibodies was restricted to the period from the 4th to the 7th week of infection, with ELISA values peaking at the 4th week. After the 6th or 7th week, the reactivity obviously decreased and remained decreased until the end of the experiment, at the 14th week.

Chronically infected rabbits were treated with praziquantel to eliminate circulating antigens (Table 1). Excretion of egg changed to negative by the 3rd week of treatment, and no worms were found at necropsy. Before treatment, antibodies were detected in only one (No. 5) out of 6 rabbits. After treatment, some negative sera changed to positive, with the positive rate rising from 16.7% to 50.0% by the 4th week. However, the ELISA value observed still remained at a low level with a considerable degree of fluctuation. No positive

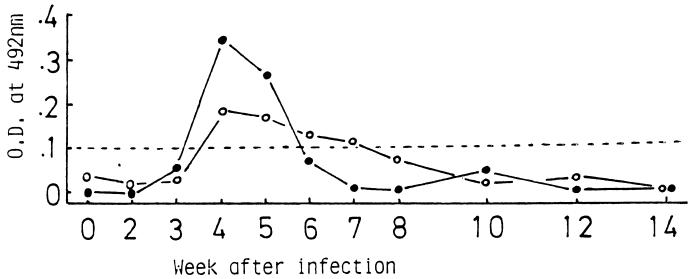

Fig. 4 Kinetic changes in levels of anti-CAA antibody in sera from *S. japonicum*-infected rabbits. The animals exposed to 50 cercariae had 15 male worms and 8 females worms (closed circle), or 10 male worms and 7 female worms (open circle), when determined at 15 weeks after infection. ELISA values higher than 0.1 (broken line) were regarded as positive.

Table 1 Changes in anti-CAA antibody levels in sera of *S. japonicum*-infected rabbits after praziquantel treatment

Rabbit no.	Age of infection (week)	Egg* no.	O.D. Before treatment	Day 1	Day 3	Week 1	Week 2	Week 3	Week 4	Week 5	Week 7	Week 9
1	23	75	.07	.04	.20†	.05	.06	.05	.21	0	0	0
2	30	9	0	0	0	.10	0	0	0	0	0	0
3	26	46	0	0	0	0	0	0	0	0	0	0
4	23	4	.06	.05	.07	.09	.16	.07	.18	.09	0	.04
5	34	51	.24	0	.32	.23	.17	.14	.20	.04	.05	.04
6	36	50	.09	.21	.08	.21	0	.17	.04	.04	.04	0
% positive			16.7	16.7	33.3	50.0	33.3	33.3	50.0	0	0	0

*; Egg no. per pellet examined before treatment.
†; Underline represents positive (>0.1)

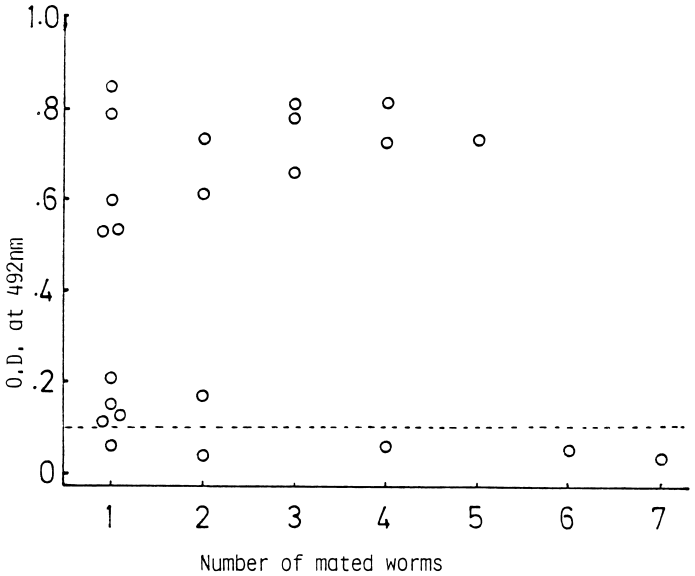

Fig. 5 Levels of anti-CAA antibody in sera from mice infected with *S. mansoni*. The animals were necropsied at 14, 27 and 44 weeks after infection. ELISA values higher than 0.1 (broken line) were regarded as positive.

reactions were seen after the 5th week of treatment.

When the reactivity of *S. japonicum*-derived CAA was studied in *S. mansoni* infection (Fig. 5), the mouse sera were found to be highly reactive with the heterologous antigen (78.2% for positive rate, Mean $0.443 \pm$ SE 0.068 for ELISA value). Furthermore, no statistical relationship was seen between ELISA values and worm burdens ($r = -0.199$).

Discussion

In this study, specific antibody response to CAA was studied using purified antigen through DEAE chromatography. Although we did not investigate the biochemical characteristics of the antigen, the minimal reaction of immune serum against *S. japonicum* egg antigens, *P. westermani,* and *G. doloresi* might indicate a high degree of purification of the antigen. In addition, our starting material, phenol extract from adult worms, has been shown to produce a single anodic band with antiserum against *S. japonicum* adult worm homogenates by immunoelectrophoresis (Hirata, 1976), suggesting that CAA is a major antigen molecule in the extract.

In the previous study with CIE, we found an inverse relationship between the incidence of antibodies and the worm burden, that is, antigen amount in the circulation (Hirata *et al.*, 1988). In the present study, we utilized ELISA to more accurately determine the relationship, and obtained similar results (Fig. 3). The relationship seems to be further supported by the results in unisexually infected mice (Fig. 2) or in relatively lightly infected rabbits (Fig. 4), since the antibody responses were higher in the circumstance that antigen amounts in the circulation are considered to be less (Hirata, 1981). These findings suggest that the detection of specific antibody against CAA might be more efficient in light or acute infection than in heavy or chronic infection.

After praziquantel treatment, a considerable portion (63%) of circulating antigen reportedly clears from the serum of *S. mansoni*-infected mice within 3 days (Weltman, 1982). In praziquantel-treated rabbits (Table 1), we expected that the elimination of parasitizing worms could result in the rise of free antibodies as

Tawfik *et al.* (1986) observed with *S. mansoni*-infected mice, which might clarify the relationship between antibodies and worm burden. However, our attempt was unsuccessful. It is speculated that a low level of antibodies and/or a comparatively short persistence of antibody production may be responsible for the results. It is unknown at what rate specific antibodies disappear. Probably many factors affect the rate: intensity or duration of infection, animal species or genetic background. Further studies are needed to clarify the cause of the present unexpected results.

This study shows that CAA extracted from *S. japonicum* adult worms is useful for sera of *S. mansoni*-infected mice. The high reactivity of *S. mansoni* sera is in agreement with previous studies (Deelder, 1973; Deelder *et al.*, 1976; Hirata *et al.*, 1988). In addition, our observation that the antibody response is unrelated to the worm burden fits with other studies with infected humans (Nash, 1978; Nash *et al.*, 1978; Nash *et al.*, 1981).

Finally, in investigations on immunological response to circulating antigens, trichloroacetic acid extract from adult worms has been the antigen material most frequently used. (Kelsoe and Weller, 1978; Qian and Deelder, 1983b; Qian and Wen, 1983). The extract from *S. mansoni* worms appears to include predominantly two major circulating antigens, CAA and cathodic antigen (CCA) (Deelder *et al.*, 1976; 1980; Nash *et al.*, 1981). On the other hand, the extract from *S. japonicum* worms is reported to include 7 circulating antigens, of which the major antigen is CAA (Qian and Deelder, 1983a). Using purified antigens from *S. mansoni.* Nash *et al.* (1981) revealed that the level of anti-GASP or CAA antibodies was high in acute and early *S. mansoni*-infected patients, and that there was no correlation between the number of eggs excreted and the antibody level, while the level of antibodies against PSAP or CCA was high in heavily chronically infected patients and correlated significantly with egg excretion. Although our employed antigen, CAA, was indicated to be highly specific, antigen characterization remains unstudied. Use of a purified antigen seems to be crucial to explore the characteristic antibody response.

Acknowledgements

The authors would like to thank the staff of the Department of Medical Zoology, Kagoshima University, for their kind supply of *S. mansoni*-infected snails.

References

1) Berggren, W. L. and Weller, T. H. (1967): Immunoelectrophoretic demonstration of specific circulating antigen in animals infected with *Schistosoma mansoni.* Am. J. Trop. Med. Hyg., 16, 606–612.
2) Deelder, A. M. (1973): Immunology of experimental infections with *Schistosoma mansoni* in the Swiss mouse and with *Fasciola hepatica* in the rabbit. Acta Leidensia, 39, 1–107.
3) Deelder, A. M., van Dalen, D. P. and van Egmond, J. G. (1978): *Schistosoma mansoni*: Microfluorometric determination of circulating anodic antigen and antigen-antibody complexes in infected hamster serum. Exp. Parasitol., 44, 216–224.
4) Deelder, A. M., de Jonge, N. Boerman, O. C., Fillie, Y. E., Hilberath, G. W., Rotmans, J. P., Gerritse, M. J. and Schut, D. W. O. L. (1989): Sensitive determination of circulating anodic antigen in *Schistosoma mansoni* infected individuals by an enzyme-linked immunosorbent assay using monoclonal antibodies. Am. J. Trop. Med. Hyg., 40, 268–272.
5) Deelder, A. M., Klappe, H. T. M., van den Aardweg, G. J. M. J. and van Meerberke, E. H. E. M. (1976): *Schistosoma mansoni*: demonstration of two circulating antigens in infected hamsters. Exp. Parasitol., 40, 189–197.
6) Deelder, A. M., Kornelis, D., van Marck, E. A. E., Eveleigh, P. C. and van Egmond, J. G. (1980): *Schistosoma mansoni*: Characterization of two circulating polysaccharide antigens and the immunological response to these antigens in mouse, hamster, and human infections. Exp. Parasitol., 50, 16–32.
7) Fujino, T., Hirata, M., Ishii, Y. and Tsutsumi, H. (1985): Immunocytochemical localization of gut-associated circulating anodic antigen in *Schistosoma japonicum.* Z. Parasitenkd., 71, 739–745.
8) Hirata, M. (1976): Circulating antigen in animals infected with *Schistosoma japonicum.* 2. Appearance of circulating antigen in infected mice. Jpn. J. Parasitol., 25, 396–401.
9) Hirata, M. (1981): Female-dependency of circulating anodic antigen level in *Schistosoma japonicum* infection. Jpn. J. Parasitol., 30, 429–437.
10) Hirata, M., Uno, M., Uno, S. and Tsutsumi, H. (1988): Correlation between worm burden and the incidence of circulating anodic antigens or antibodies

in mice infected with *Schistosoma japonicum* or *Schistosoma mansoni*. Kurume Med. J., 35, 13–18.
11) Kelsoe, G. H. and Weller, T. H. (1978): Immunodiagnosis of infection with *Schistosoma mansoni*: enzyme-linked immunosorbent assay for detection of antibody to circulating antigen. Proc. Natl. Acad. Sci., 75, 5715–5717.
12) Lichtenberg, F. von, Bawden, M. P. and Shealey, S. H. (1974): Origin of circulating antigen from the schistosome gut. An immunofluorescent study. Am. J. Trop. Med. Hyg., 23, 1088–1091.
13) Nash, T. E. (1974): Localization of the circulating antigen within the gut of *Schistosoma mansoni*. Am. J. Trop. Med. Hyg., 23, 1085–1087.
14) Nash, T. E. (1978): Antibody response to a polysaccharide antigen present in the schistosome gut. I. Sensitivity and Specificity. Am. J. Trop. Med. Hyg., 27, 938–943.
15) Nash, T. E., Lunde, M. N. and Cheever, A. W. (1981): Analysis and antigenic activity of a carbohydrate fraction derived from adult *Schistosoma mansoni*. J. Immunol., 126, 805–810.
16) Nash, T. E., Nasir-Ud-Din and Jeanloz, R. W. (1977): Further purification and characterization of a circulating antigen in schistosomiasis. J. Immunol., 119, 1627–1633.
17) Nash, T. E., Ottesen, E. A. and Cheever, A. W. (1978): Antibody response to a polysaccharide antigen present in the schistosome gut. II. Modulation of antibody response. Am. J. Trop. Med. Hyg., 27, 944–950.
18) Nash, T. E., Prescott, B. and Neva, F. A. (1974): The characteristics of a circulating antigen in schistosomiasis. J. Immunol., 112, 1500–1507.
19) Qian, Z. L. and Deelder, A. M. (1983a): *Schistosoma japonicum*: Immunological characterization and detection of circulating polysaccharide antigens from adult worms. Exp. Parasitol., 55, 168–178.
20) Qian, Z. L. and Deelder, A. M. (1983b): *Schistosoma japonicum*: Immunological response to circulating polysaccharide antigens in rabbits with a light infection. Exp. Parasitol., 55, 394–403.
21) Qian, Z. L. and Wen, H. C. (1983): Schistosome circulating antigens (CSA) as a possible diagnostic parameter for active infections. Acta Leidensia, 51, 37–52.
22) Tawfik, A. F., Carter, C. E. and Colley, D. G. (1986): Effects of anti-schistosomal chemotherapy on immune responses, protection and immunity. I. Changes in cellular and humoral responses. Am. J. Trop. Med. Hyg., 35, 100–109.
23) de Water, R., Fransen, J. A. M. and Deelder, A. M. (1986): Ultrastructural localization of the circulating anodic antigen in the digestive tract of *Schistosoma mansoni* using monoclonal antibodies in an immunogold labeling procedure. Am. J. Trop. Med. Hyg., 35, 549–558.
24) Weltman, J. K. (1982): Effect of praziquantel on antigenemia in murine schistosomiasis. Am. J. Trop. Med. Hyg., 31, 1294–1296.