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# *Angiostrongylus cantonensis* Infection in Immunosuppressed Mice

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## Abstract

Mice from each of the following strains; BALB/c, CBA, A/J, C57BL/6 and C57BL/10, were divided into two groups by the numbers of *Angiostrongylus cantonensis* larvae (20 or 30) given to each mouse. Both groups of mice were further divided into control and immunosuppressed subgroups containing 15 mice in each. A single dose of 0.05 ml of cortisone acetate suspension was injected subcutaneously into the mice every other day for a total of three injections. The results showed that the average survival time in the immunosuppressed mice was significantly longer than in the control mice. The number of worms found in immunosuppressed mice was also significantly higher than those found in control mice. The average size of male and female worms recovered from immunosuppressed mice was also bigger than those from control mice. The body weights of all mice decreased gradually, and significant weight loss occurred in the third week after infection. However, with the exception of $B_{10}$ mice, the mean body weight loss in each control group was similar to that of the immunosuppressed group. The results were dependent on the number of larvae given.

**Key words:** *Angiostrongylus cantonensis*, infection, immunosuppressed mouse

## Introduction

*Angiostrongylus cantonensis* is a rat-lung worm generally considered to be the etiological agent of human eosinophilic meningitis or meningoencephalitis in Southeast Asian countries and the Pacific Islands (Alicata and Jindrak, 1970). Molluscan intermediate hosts and paratenic hosts are involved in the life cycle of this worm (Chen, 1979). In rats, the ingested infective third-stage larvae penetrate the intestinal wall and migrate to the brain and spinal cord where they moult and develop into their juvenile stage. Subsequently, these worms return to pulmonary arteries and lungs where they reach sexual maturity and lay eggs (Mackerras and Sandars, 1955; Bhaibulaya, 1975).

Acquired resistance to this parasite has been observed in naturally infected wild rats and has also been demonstrated experimentally (Yoshimura *et al.*, 1979). Humoral immune response to *A. cantonensis* has been detected in human cases and in experimentally infected animals by various immunodiagnostic techinics (Welch, *et al.*, 1980; Cross, 1982; Yong and Dobson 1982; Tharavanij, 1979). Cell-mediated immune response also becomes measurable in rats several weeks after infection. Significant responses were noticed in the peripheral blood lymphocytes of infected rats during the period of 5 to 10 weeks of infection (Yoshimura and Soulsby, 1976). Induction of protective immunity against lethal challenge of infective larvae has been achieved in rats by immunization with irradiated third-stage larvae or excretory and secretory products from adult female worms (Lee, 1969; Dharmkrong-AT *et al.*, 1978; Techasoponmani and Sirisinha, 1980). On the contrary, heavier infections has been noticed in splenectomized rats and neonatally thymectomized mice (Yoshimura *et al.*, 1982; Yong *et al.*, 1983). It is undoubted that antigens of *A. cantonensis* may invoke the hosts to produce antibody that plays an important role in protecting themselves from superinfection by this worm.

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Corticosteroids are popularly used in modern medicine to treat or control diseases. Many populations have the opportunity to be prescribed this kind of drug. The present study is an attempt to understand the possibility of severe infection of this worm occurring in immunosuppressed animals induced by corticosteroids as it is related to the infection in humans. As the non-permissive host, five strains of mice were used.

## Materials and Methods

1. Preparation of third-stage larvae

The third-stage larvae were collected from naturally infected *Achatina fulica*, the main intermediate host in southern Taiwan. The snails were minced by blender (Cole-Parmer Instrument Co.) and digested in magnetically stirred digestive fluid (1:10,000 pepsin, 2 g; conc. Hcl, 7 ml; Dist. Water, 1,000 ml) at room temperature for 4 hours. The suspension was washed according to the following procedure; two-thirds of the solution was discarded after standing 15 minutes and was then filled with tap water several times. The third-stage larvae-rich precipitated solution was used to fill in the penetration apparatus as shown in Fig. 1. After standing for 1 to 2 hours, larvae which penetrated through the wipe paper to the bottom of the petri dish were collected and identified under the stereomicroscope.

2. Experimental animals

Three- to four-month old mice, BALB/c, CBA, A/J, C57BL/6 ($B_6$) and C57BL/10 ($B_{10}$) strains, were used in the present study. Sixty mice of each strain were evenly divided into two groups according to the number of larvae, 20 or 30, used to infect each mouse. Both groups of mice were further divided into immunosuppressed and control subgroups i.e., 15 mice involved in each subgroup.

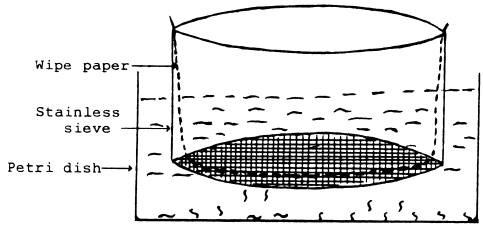


Fig. 1 Apparatus used for the collection of *Angiostrongylus cantonensis*. (Larvae-rich precipitated fluid filled in the stainless sieve)

3. Experimental design

The body weight of every mouse was measured and recorded before and every week after infection. Ten uninfected mice of each strain were also weighed as a control for body weight gain or loss. A single dose of 0.05 ml of cortisone acetate suspension (Schering Pharmaceutical Co.) was injected subcutaneously every other day for three times to induce the immunosuppressive status. The day after the last injection, immunosuppressed and control mice were infected with third-stage larvae of *A. cantonensis* by a stomach tube. Twenty sera specimens from corticosteroid-treated and control BALB/c and $B_6$ strains mice, infected with 30 larvae each, were examined 28 days after infection for humoral immune response by ELISA described elsewhere (Yen *et al.*, 1984). Briefly, the ELISA procedure was performed as follows. Specific antibody in serum were first combined with coated antigen and then the second antibody conjugated with enzyme sequentially reacted with specific antibody. The optical density of the substrate solution added in the well was read out by Multiskane (Flow Co.) at 490 nm wave length. Horseraddish peroxidase conjugated with goat anti-mouse IgG, IgA, IgM, IgE and complement (Nordiac Co.) was used in this study.

Mice were given sufficient food and water in animal cages in an air conditioned room after infection. The survival time (days) of each mouse was carefully observed every day up to the thirtieth day after infection. The skulls of 2 mice, in each subgroup, were opened and the brains were removed on the twenty-first day after infection. Juvenile worms were collected by teasing brain tissue with a needle under a dissecting microscope. Besides the brains, the visceral organs including liver, lung and heart

of each mouse were also examined for worms. The number and length of each male and female worm was counted and measured under a microscope with a micro-scale apparatus. All other mice remaining in each subgroup were examined at death and the number of worms were counted.

Mean body weight loss in the third week after infection, survival days, and the number and length of juvenile worms were compared between the immunosuppressed and the control group mice depending on the infection dose.

Statistical analyses between each paired subgroup were done by Student's *t*-tests, when $p < 0.05$ showed a significant difference.

## Results

1. Serum antibody

Humoral antibodies in corticosteroid treated and control BALB/c and $B_6$ strain mice were detected by ELISA. The results are shown in Table 1. The IgA, IgE and complement antibodies were very similar between the corticosteroid treated and control groups of each strain of mice. Both IgM and IgG antibodies in the corticosteroid treated BALB/c strain mice, 0.37 and 0.64 respectively, were lower than 0.46 and 0.92 respectively in the control mice. The IgM and IgG antibodies in the immunosuppressed $B_6$ mice were also lower than in the control mice.

2. Survival time

Both immunosuppressed and control mice of all strains remained alive at the end of observation in the group given 20 larvae each with the exception of the BALB/c strain, in which the mean survival time of immunosuppressed and control mice, as shown in Table 2, was 28.7 respectively.

In the group where each mouse was infected

Table 1 ELISA titers in corticosteroid treated and control mice 28 days after infected with *A. cantonensis*

| Mouse strain | Corticosteroid treated | IgG | IgA | IgM | IgE | Complement |
|---|---|---|---|---|---|---|
| BALB/c | − | 0.92±0.05* | 0.20±0.07 | 0.46±0.07‡ | 0.17±0.03 | 0.27±0.06 |
| BALB/c | + | 0.64±0.05* | 0.20±0.03 | 0.37±0.04‡ | 0.14±0.02 | 0.24±0.02 |
| B6 | − | 0.96±0.05† | 0.23±0.01 | 0.50±0.01§ | 0.18±0.02 | 0.29±0.01 |
| B6 | + | 0.84±0.04† | 0.21±0.05 | 0.30±0.03§ | 0.15±0.01 | 0.25±0.01 |

Elisa titers expressed as optical densities ($\bar{X}$ ±S.D.).
*, †, ‡, §: Student's *t*-test, $p < 0.01$.

Table 2 Worm recovery and survival day of 5 different strains mice infected with 20 third-stage *Angiostrongylus cantonensis* larvae

| Mouse strain | No. mouse | Worm recovery*† | | Survival day* | |
|---|---|---|---|---|---|
| | | Immuno-suppress | Control | Immuno-suppress | Control |
| BALB/c | 15 | 14.8±0.8 | 11.4±0.9 | 28.7±2.6 | 28.7±1.3 |
| CBA | 15 | 13.8±1.2 | 10.0±2.2 | 30 | 30 |
| A/J | 15 | 13.8±2.1 | 9.6±1.7 | 30 | 30 |
| $B_6$ | 15 | 12.8±1.1 | 8.2±2.5 | 30 | 30 |
| $B_{10}$ | 15 | 12.3±1.9 | 7.0±2.0 | 30 | 30 |

*Expressed as $\bar{X}$ ±S.D.
†Student's *t*-test for mean worm recovery from immunosuppressed versus control group of each strain, $p < 0.01$.

with 30 larvae, as shown in Table 3, the mean survival time for immunosuppressed mice was shorter than for control mice of BALB/c, CBA and A/J strains. The survival time in both the immunosuppressed and the control groups of $B_6$ and $B_{10}$ strain mice was more than 30 days.

3. Recovery of worms

All juvenile worms were collected from the brain when the mouse was dead. No worm was found in other visceral organ. In the group given 20 larvae each, the mean number of worms found in immunosuppressed and control mice, as shown in Table 2, in the BALB/c strain, was 14.8 and 11.4 respectively. This was the highest number. In $B_{10}$ strain, 12.3 and 7.0 were found respectively, this being the least number found. In the group given 30 larvae each, the mean number of worms recovered from immunosuppressed and control mice, as shown in Table 3, ranged from 24.6 in the CBA strain to 19.8 in the $B_{10}$ strain and 18.8 in the BALB/c strain to 10.4 in the $B_{10}$ strain respectively.

4. Measurement of mice weight

The loss of body weight in all mice infected with 20 and 30 larvae is shown in Tables 4 and 5. The average body weight decreased more than 4.0 gm in all immunosuppressed and control mice, except the $B_{10}$ strain, on the third week after infection. The average body weight loss in immunosuppressed and control $B_{10}$ strain mice was 4.5 and 2.2 gm in the group given 20 larvae, and 4.2 and 2.4 gm in the group given 30 larvae. However, a slight

Table 3 Worm recovery and survival day of 5 different strains mice infected with 30 third-stage *Angiostrongylus cantonensis*

| Mouse strain | No. mouse | Worm recovery*† | | Survival day | |
|---|---|---|---|---|---|
| | | Immuno-suppress | Control | Immuno-suppress | Control |
| BALB/c | 15 | 23.8±1.2 | 18.8±3.4 | 23.1±1.6 | 23.2±4.1 |
| CBA | 15 | 24.6±1.6 | 16.3±2.6 | 24.6±2.7 | 26.7±3.7 |
| A/J | 15 | 20.0±2.4 | 15.6±3.4 | 28.7±2.2 | 29.1±3.1 |
| $B_6$ | 15 | 21.6±1.7 | 11.2±3.6 | 30 | 30 |
| $B_{10}$ | 15 | 19.8±1.1 | 10.4±2.5 | 30 | 30 |

*Expressed as $\bar{X}$ ±S.D.

†Student's *t*-test for mean worm recovery from immunosuppressed versus control group of each strain, $p < 0.01$.

Table 4 Average body weight (gm) loss in 5 different strains of mice after infected with 20 third-stage *Angiostrongylus cantonensis* larvae

| Mouse Strain | 1st week | | 2nd week | | 3rd week | |
|---|---|---|---|---|---|---|
| | IMS | CTL | IMS | CTL | IMS | CTL |
| BALB/c | 0.5* | 0.4 | 1.2 | 1.4 | 5.7 | 5.6 |
| CBA | 0.1* | 0.4 | 1.4 | 0.9 | 5.2 | 5.2 |
| A/J | 0.4 | 0.6 | 1.3 | 1.2 | 5.0 | 5.6 |
| $B_6$ | 0.1 | 0.1 | 2.1 | 0.2 | 5.3 | 4.2 |
| $B_{10}$ | 0.5 | 0.4* | 1.6 | 0.2* | 4.5 | 2.2 |

IMS : Immunosuppressed mice.
CTL : Control mice.
* Increase of Body weight.

Table 5 Average body weight (gm) loss in 5 different strains of mice after infected with 30 third-stage *Angiostrongylus cantonensis* larvae

| Mouse Strain | 1st week | | 2nd week | | 3rd week | |
|---|---|---|---|---|---|---|
| | IMS | CTL | IMS | CTL | IMS | CTL |
| BALB/c | 0.4 | 0.3 | 1.5 | 1.5 | 6.4 | 5.7 |
| CBA | 0.6 | 0.6 | 2.2 | 1.6 | 6.0 | 5.3 |
| A/J | 0.2 | 0.1 | 2.2 | 0.8 | 5.0 | 5.1 |
| $B_6$ | 0.4 | 0.3 | 1.7 | 0.8 | 4.6 | 4.3 |
| $B_{10}$ | 0.5 | 0.3 | 2.5 | 0.3 | 4.2 | 2.4 |

IMS : Immunosuppressed mice.
CTL : Control mice.

increase in body weight was observed in uninfected control mice of each strain during the same week.

5. Size of worms

In all strains of mice, the mean length of male and female juvenile worms from immunosuppressed mice infected with 20 larvae, was longer than from control mice as shown in Table 6. In the group where 30 larvae were given to each mouse, the mean length of worms from immunosuppressed mice was also longer

Table 6 Length (cm) of juvenile worms from 5 different strains mice infected with 20 third-stage *Angiostrongylus cantonensis* larvae

| Mouse Strain | Immunosuppressed mice | | Control mice | |
|---|---|---|---|---|
| | Male worms | Female worms | Male worms | Female worms |
| BALB/c | 0.98±0.1 (18) | 1.01±0.1 (19) | 0.90±0.1 (17) | 0.93±0.1 (18) |
| CBA | 1.01±0.1 (14) | 1.15±0.1 (15) | 0.91±0.1 (10) | 1.01±0.2 (10) |
| A/J | 1.02±0.1 (14) | 1.09±0.1 (18) | 0.89±0.2 (12) | 0.92±0.2 (12) |
| $B_6$ | 0.87±0.1 (10) | 0.98±0.2 (15) | 0.78±0.1 (11) | 0.82±0.1 (10) |
| $B_{10}$ | 0.85±0.1 (12) | 0.99±0.1 (12) | 0.73±0.1 (10) | 0.80±0.2 (10) |

( ) Number of worm measured.
Student's *t*-test for mean length of male and female worms from immunosuppressed mice versus those from control mice, $p < 0.01$.

Table 7 Length (cm) of juvenile worms from 5 different strains mice infected with 30 third-stage *Angiostrongylus cantonensis* larvae

| Mouse Strain | Immunosuppressed mice | | Control mice | |
|---|---|---|---|---|
| | Male worms | Female worms | Male worms | Female worms |
| BALB/c | 1.05±0.1 (15) | 1.18±0.1 (19) | 0.92±0.1 (11) | 0.95±0.1 (21) |
| CBA | 1.06±0.1 (22) | 1.13±0.1 (18) | 0.90±0.2 (10) | 1.00±0.2 (11) |
| A/J | 1.03±0.1 (14) | 1.16±0.1 (23) | 0.86±0.2 (14) | 0.96±0.3 (10) |
| $B_6$ | 0.94±0.2 (13) | 1.08±0.1 (21) | 0.71±0.2 ( 7) | 0.81±0.1 ( 6) |
| $B_{10}$ | 0.89±0.1 (18) | 0.94±0.1 (17) | 0.72±0.3 (10) | 0.79±0.2 (12) |

( ) Number of worm measured.
Student's *t*-test for mean length of male and female worms from immunosuppressed mice versus those from control mice, $p < 0.01$.

in each strain of mice as shown in Table 7.

## Discussion

The infection of *A. cantonensis* in five strains of immunosuppressed mice induced by corticosteroid without any deficiency in organs involved in immune response was observed in this study. In this study, significantly lower specific IgM and IgG levels against *A. cantonensis* antigen were observed in corticosteroid treated immunosuppressed mice after infection of this worm. The average number of worms recovered from five strains of immunosuppressed mice infected with either 20 or 30 third-stage *A. cantonensis* larvae was significantly higher than the number recovered from control mice (Student's *t*-test, $p < 0.01$ in each pair of mice). This corresponds with the report of Yong *et al.* (1983), that worms recovered from splenectomized rats were more than those obtained from intact rats.

All except BALB/c strain mice were lived more than 30 days after being infected with 20 third-stage *A. cantonensis* larvae. The more larvae given, the shorter the survival time observed. The mean survival days in both immunosuppressed and control BALB/c strain mice infected with 30 *A. cantonensis* larvae was statistically less than in those infected with 20 *A. cantonensis* larvae (Student's *t*-test, $p < 0.01$). Similar results were also obtained in CBA strain mice. The survival days could not be compared in $B_6$ and $B_{10}$ strains mice between two different infection dose, either immunosuppressed or control groups, because those mice were still alive at the end of observation.

Although significantly more worms were collected from immunosuppressed mice than those from control mice infected with either 20 or 30 larvae, the survival days were not significantly different between immunosuppressed and control mice of each strain of mice. It is suggested that the infection dose of 30 third-stage larvae was lethal to BALB/c, CBA and A/J strain mice. Neither immunosuppressed nor control mice lived more than 30 days after exposure to that infection dose. However, $B_6$ and $B_{10}$ strain mice well tolerated this infection dose. Hence, they did not demonstrate any significant difference in the number of survival days after infection.

Three-to four-month old mice are physically matured and their body weights were at a stable level in uninfected mice, but all infected mice lost body weight gradually with obviously increased weight loss, occurring in the third week after infection. The mean body weight loss in each strain of mice in the control group, except $B_{10}$ strain mice, was similar to that of those in the immunosuppressed group 3 weeks after infection with 20 or 30 larvae. Loss of appetite was observed and became severe in the third week in infected mice. This may have been due to eosinophilic meningitis.

The length of juvenile worms from human patients or laboratory infected rats have been measured in some literature (Hwang *et al.*, 1984; Uahkowitchai *et al.*, 1977; Yong and Dobson, 1982; Yong *et al.*, 1983). Yong *et al.* (1983) indicated most female *A. cantonensis* worms recovered from splenectomized rats 42 days after infection were longer than those from intact and sham operated rats. Yoshimura *et al.* (1982) reported mean body length of worms recovered from BALB/c athymic nude mice were also longer than those from heterozygous littermates BALB/c mice. It showed the thymus- and spleen-dependent immune systems appeared to be definitely involved in the retardation of normal worm development. Makinodan *et al.* (1970) reported several kinds of drugs and hormones may be used to effect suppression of the immune response. Hormones, particularly the corticosteroids, act nonspecifically on immune reaction in intact hosts (Parker and vaura, 1974; Webb, 1976). Male and female juvenile worms from immunosuppressed mice induced by corticosteroid were shown significantly longer than those from control mice in this study (Student's *t*-test, $p < 0.01$).

Hundreds of human cases with eosinophilic meningitis have been reported in Taiwan, some of which have resulted in the death of the patients (chen, 1979; Hwang *et al.*, 1986). Ko

(1978) accidentally observed that *A. cantonensis* invaded the heart of a immunosuppressed spider monkey induced by dexamethasone for ulcer treatment of the monkey. One human case involving lung angiostrongyliasis in Taiwan was described by Yii *et al.* (1968) with the status of immunosuppression. *A. cantonensis* infectivity in mice demonstrated some differences between the immunosuppressed and control groups. Higher worm recovery rates, shorter survival periods and larger worm size were observed in the immunosuppressed mice in the present study. It is possible that more worms are harboured in the brain and the spinal cord of immunosuppressed patients and that may cause more severe pathological changes leading to death.

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**References**

1) Alicata, J. E. and Jindark, K. (1970): Angiostrongylosis in the Pacific and Southeast Asia. Charles C. Thomas, Springfield, Illinois, 105.
2) Bhaibulaya, M. (1975): Comparative studies on the life history of *Angiostrongylus mackerrasae* Bhaibulaya, 1968 and *Angiostrongylus cantonensis* (Chen, 1935). Int. J. Parasitol., 5, 7–20.
3) Chen, E. R. (1979): Angiostrongyliasis and eosinophilic meningitis on Taiwan: A review. In "Studies on angiostrongyliasis in Eastern Asia and Australia", J. H. Cross (Editor). NAMRU-2 Special Publication No. 44, Taipei, Taiwan, 57–73.
4) Cross, J. H. and Chi, C. H. (1982): ELISA for the detection of *Angiostrongylus cantonensis* antibodies in patients with eosinophilic meningitis. Southeast Asian J. Trop. Med. Pub. Hlth., 13, 73–76.
5) Dharmkrong-AT, A., Uahkowitchai, V. and Sirisinha, S. (1978): The humoral and cell-mediated immune responses to somatic and metabolic antigens in rats infected with *Angiostrongylus cantonensis*. Southeast Asian J. Trop. Med. Pub. Hlth., 9, 330–337.
6) Hwang, K. P., Huang, C. T., Chen, T. S., Chen, E. R., Shih, C. C. and Hsieh, H. C. (1984): Measurements of *Angiostrongylus cantonensis* discovered from human cases. Proceedings of Sino-Japanese Symposium on Parasitic Zoonoses. Kaohsiung, Taiwan, R. O. C., 124–129.
7) Hwang, K. P., Chen, E. R., Yen, C. M. and Hsieh, H. C. (1986): An outbreak of angiostrongyliasis in a family in Taiwan. Proceedings of Sino-Japanese Symposium on Parasitic Zoonoses. Osaka, Japan. 165–171.
8) Ko, R. C. (1978): Occurrence of *Angiostrongylus cantonensis* in the heart of a spider monkey. J. Helminthol., 52, 229.
9) Lee, S. H. (1969): The use of irradiated third-stage larvae of *Angiostrongylus cantonensis* as antigen to immunize albino rats against homologous infection. Proc. Helminth. Soc. Wash., 36, 95–97.
10) Mackerras, M. J. and Sandars, D. F. (1955): The life-history of the rat lungworm, *Angiostrongylus cantonensis* (Chen) Nematoda: Metastrongylidae. Australian J. zoology., 3, 1–21.
11) Makinodan, T., Santos, G. W. and Quinn, R. P. (1970): Immunosuppressive drugs. Pharm. Rev., 22, 189–247.
12) Parker, C. W. and Vaura, J. D. (1974): Immunosuppression. Prog. Hematol., 6, 1, 1974.
13) Techasoponmani, R. and Sirisinha, S. (1980): Use of excretory and secretory products from adult female worms to immunize rats and mice against *Angiostrongylus cantonensis* infections. Parasitology, 80, 457–469.
14) Tharavanij, S. (1979): Immunology of angiostrongyliasis. In "Studies on angiostrongyliasis in Eastern Asia and Australia", J. H. Cross (Editor). NAMRU-2 Special Publication No. 44, Taipei, Taiwan, 151–164.
15) Uahkowithchai, V., Techasoponmani, R. and Sirisinha, S. (1977): Effect of immunization with metabolic antigens on experimental infection of rats with *Angiostrongylus cantonensis*. Southeast Asian J. Trop. Med. Pub. Hlth., 8, 486–493.
16) Webb, D. R. (1976): Immunosuppression and immunopotentiation. In "Basic and Chinical Immunology" edited by Fudenberg, H. H., Sites, D. P., Caldwell, J. L. and Wells, J. V. Lange Medical Publical Publications, Los Altos, California, 260–266.
17) Welch, J. S., Dobson, C. and Campbell, G. R. (1980): Immunodiagnosis and seroepidemiology of *Angiostrongylus cantonensis* zoonoses in man. Tran. Roy. Soc. Trop. Med. Hyg., 74, 614–623.
18) Yen, C. M., Chen, E. R., and Hsieh, H. C. (1984): Immunodiagnosis of human clonorchiais by a micro-enzyme linked immunosorbent assay. Chinese J. Microbiol Immunol. 17; 34–42, 1984.
19) Yii, C. Y., Chen, C. Y., Fresh, J. W., Chen, T. and Cross, J. H. (1968): Human angiostrongy-

liasis involving the lungs. Chinese J. Microbiol., 1, 148–150.
20) Yong, W. K. and Dobson, C. (1982): Antibody responses in rats infected with *Angiostrongylus cantonensis* and the passive transfer of protective immunity with immune serum. Z. Parasitenkd., 67, 329–336.
21) Yong, W. K., Glanville, R. J. and Dobson, C. (1983): The role of the spleen in protective immunity against *Angiostrongylus cantonensis* in rats: Splenectomy and passive spleen cell transfers. International J. Parasitol., 13, 165–170.
22) Yoshimura, K. and Soulsby, E. J. L. (1976): *Angiostrongylus cantonensis*: Lymphoid cell responsiveness and antibody production in rats. Am. J. Trop. Med. Hyg., 25, 99–107.
23) Yoshimura, K., Aiba, H. and Oya, H. (1979): Transplantation of young adult *Angiostrongylus cantonensis* into the rat pulmonary vessels and its application to the assessment of acquired resistance. Int. J. Parasitol., 9, 97–103.
24) Yoshimura, K., Sato, K., Uchida, K. and Oya, H. (1982): The course of *Angiostrongylus cantonensis* infection in athymic nude and neonatally thymectomized mice. Z. Parasitenkd, 67, 217–226.