[Jpn. J. Parasitol., Vol. 45, No. 2, 85–92, April, 1996]

Physiological Reactions Induced by Larval *Ascaris suum* Infection

TOSHINORI KOMATSU[1)], ATSUSHI YAMATODANI[4)], KUMIKO NAGATA[2)], HARUKI OKAMURA[2)], TOSHIHIDE TAMURA[2)], KAZUO NAGAI[3)], NORIATU SAEKI[1)] AND SOHEI SHINKA[1)]

[1)]*Department of Immunology and Medical Zoology,* [2)]*Department of Bacteriology and* [3)]*Department of Pharmacology, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya, Hyogo 663 Japan.* [4)]*Department of Medical Physics, School of Allied Health Sciences, Faculty of Medicine, Osaka University, Yamadaoka 2-2, Suita, Osaka 565, Japan.*

(Accepted February 9, 1996)

Abstract

Effects of the infection with larval *A. suum* on the immune system seem to be primarily induced by the tissue injury of target organs. In this study, the tissue damage caused by the larval migration was substantiated by measuring enzymatic activities. The activities of both transaminases, GOT and GPT in sera of infected mice peaked on the 5th day of infection when the number of larvae in the liver was maximal. The total activities of histidine decarboxylase (L-histidine carboxylase, EC 4.1.1.22) (HDC) in the liver or in the lung increased when the larvae migrated to respective organs. The infected mice normally recovered from the debility after the remove of larvae from the lung. However, the administration of a histamine H_2-receptor antagonist, famotidine, interfered with the recovery. In the circulation, the corticosterone level significantly increased from the 3rd day and peaked on the 7th day of infection ($p<0.01$). On the other hand, catecholamines (adrenalin, noradrenalin and dopamine) significantly decreased to below normal levels on the 8th day ($p<0.02$, 0.05 and 0.01, respectively). These data suggest that the systemic feedback regulation with corticosterone that is induced by local tissue damages may affect the immune system.

Key words: larval *Ascaris suum* infection; glutamic oxaloacetic transaminase; glutamic pyruvic transaminase; histidine decarboxylase; corticosterone; catecholamine.

Introduction

The larvae of *Ascaris suum* migrate through the liver and lung in mice, resembling that in its definitive host, the swine and being synchronous (Sprent, 1952; Mitchell *et al.*, 1976). Our study have shown that the infection largely affects the murine immune system (Komatsu *et al.*, 1996). Although the infection induces the bulk of immunoglobulin synthesis after the remove of larvae from the lung, it suppresses antibody responses against unrelated antigens administered a few days before or after the infection (Crandall *et al.*, 1971; Crandall *et al.*, 1976; Komatsu *et al.*, 1996). The dramatic change in the composition of splenocytes seems to reflect the abnormality in immune responses.

The immune system is bi-directionally linked with the nervous and endocrine systems (Blalock, 1989; Goetzl *et al.*, 1990). Therefore, the immunological phenomena seen in the host infected with parasites should be generally considered as results of systemic, physiological reactions. Stimuli that induce physiological reactions are integrated as stress. Stresses modulate immune responses in various ways depending on the nature and intensity of the stimuli (Stein *et al.*, 1985). However, the intense stress such as tissue damage generally induces immunosuppression through the release of glucocorticoids (Freire-Garabal *et al.*, 1991; Munck *et al.*, 1984; Stein *et al.*, 1985).

The helminth infection is considered as a stressful event probably with both cognitive and noncognitive stimuli. The cognitive stimuli may be generated by dynamic movement. Although it is

Correspondence: Toshinori Komatsu

小松俊憲[1]，大和谷　厚[4]，長田久美子[2]，岡村春樹[2]，田村俊秀[2]，永井和男[3]，佐伯典厚[1]，新家荘平[1]

([1]兵庫医科大学免疫医動物学教室，[2]兵庫医科大学細菌学教室，[3]兵庫医科大学薬理学教室，[4]大阪大学医学部保健学科医用物理学教室)

difficult to assess the sole effect of the cognitive stimuli, some products resulting from tissue damages or subsequent inflammatory reactions can stimulate the central nervous system. Noncognitive stimuli such as antigens are trapped by immune systems. The latter stimuli may be also transmitted to the neuroendocrine system through the production of cytokines and peptide hormones when the production is serious (Blalock, 1989; Goetzl *et al.*, 1990).

In mice infected with *A. suum* larvae, the thymic atrophy and the change in cellular composition are seen together with the splenic perturbation. This suggests the operation of systemic feedback regulation. Our studies on analyses of kinetics of cellular or humoral parameters have suggested that the regulation may be primarily induced by the tissue damage of target organs.

In this study, we present data showing the abnormality in target organs by means of enzymic activities such as transaminases and HDC. We also show the change in corticosterone and catecholamine levels in the circulation as the proof of the operation of feedback regulation.

Materials and Methods

Animals

Female BDF1 mice at 7 weeks of age were obtained from Shizuoka Laboratory Animal Center (Hamamatsu, Japan).

Infection with Ascaris suum *larvae*

The coats of fertilized eggs obtained from the uterus of adult *Ascaris suum* were removed with 10% NaOCl and incubated at 28°C for 4 weeks. Mice were infected with 1×10^4 embryonated eggs each by stomach intubation.

Serum and plasma

To assay transaminases, serum was separated from blood taken by heart puncture. To assay corticosterone and catecholamines, heparinized blood was collected in the absence (corticosterone) or in the presence (catecholamines) of sodium metabisulfite (0.5 mg/ml) from guillotined mice and immediately centrifuged at 4°C. Serum and plasma samples were frozen until use.

Plasma catecholamines

Nine hundred microliters of 3% perchloric acid containing 2 mM EDTA-2Na and $Na_2S_2O_5$ was added to 100 μl of plasma, then the mixture was centrifuged at 10,000×g for 30 min at 4°C. Adrenaline, noradrenaline and dopamine in 500 μl of the deproteinized plasma were determined using a fully automated HPLC-fluorometric system (HLC-8030 Catecholamine Analyzer, Tosoh, Tokyo, Japan), by means of a diphenylethylenediamine condensation method (Nohta *et al.*, 1984).

Corticosterone

The corticosterone concentration in the plasma was determined by radioimmunoassay using a ^{125}I corticosterone kit specifically designed for murine plasma (ICN Biomedicals, Inc., Costa Mesa, CA). In the assay, the reaction between a limited amount of rabbit anti-corticosterone antibody and the corresponding hormone labeled with a radioisotope was inhibited with the unlabeled hormone in test samples.

Transaminases

Activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured by means of the POP. TOOS method (pyruvate oxidase, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine and 4-aminoantipyrine) using kit (Transaminase CII-test, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Activities are expressed as Karmen units.

Histidine decarboxylase activity

HDC activities of whole liver and lung from BDF1 mice infected with *A. suum* larvae were assayed as described (Kawaguchi-Nagata *et al.*, 1988). Briefly, the organs were homogenized in cold solution A (0.1 M potassium phosphate buffer, pH 6.8, 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate, 1% polyethylene glycol 300, and 0.5 μg/ml each of leupeptin and chemostatin). The homogenate (final volume of 3 ml) was centrifuged at 10000×g for 20 min and the supernatant was dialyzed extensively against solution A at 4°C. The dialysate (0.5 ml) was incubated with an equal volume of 0.25 mM L-histidine or solution A at 37°C for 15 h, then a small volume of perchloric acid (finally 3%) was

added to the reaction mixture. Histamine in the supernatant was separated from histidine and measured fluorometrically using o-phthalaldehyde in an HPLC system as described (Yamatodani *et al.*, 1985).

Statistical analyses

Data are expressed as the means and standard errors. The significance of the data was evaluated by Student's t-test.

Results

Transaminase activities

Transaminase activities in sera of mice infected with 1×10^4 embryonated eggs of *A. suum* were assayed colorimetrically (Fig. 1). The normal levels of GOT and GPT activities were 148.8±22.5 and 21.1±1.5 Karmen units, respectively. Both enzymatic activities increased in parallel from the 3rd day of infection and peaked on the 5th day. The peak activities of GOT and GPT were 642.3±121 and 557.6±156 units, respectively. The 5th day of infection corresponded with the time when the concentration of larvae in the liver was maximal. The remarkable increases in both enzymatic activities were transient and returned to the normal levels on the 8th (GOT) or 10th days (GPT) after the infection. The statistical significance of the increases in GOT activity from the 3rd to the 7th day was $0.01<p<0.05$ and that in GPT activity from the 5th to the 7th day was $P<0.01$ or $P<0.05$.

Histidine decarboxylase activities

The total HDC activities in the whole liver and lung of infected mice were assayed and expressed as picomols per minute per organ (pmol/min/organ) (Fig. 2). Each value is the mean of 3 results and the standard error. The activities in the normal liver and lung were 0.156±0.049 and 0.191±0.016 pmol/min/organ, respectively. The levels in both organs increased from the 4th day of infection. However, the enzymatic activity in the liver peaked on the 6th day of infection, whereas that in the lung peaked on the 8th day of infection. The peak levels were 1.960±0.225 pmol/min/organ ($p<0.01$) in the liver and 6.570±1.753 pmol/min/organ ($p<0.1$) in the lung. Therefore, HDC activity of the latter organ was elevated about 34-fold by the infection. Even on the 13th day, the activity (3.757±0.798 pmol/min/organ at $p<0.05$) was still high in this organ. To confirm that the assayed activities were those of endogenous HDC, the extract from 1×10^5 embryonated eggs was tested by the same means. No activity was detected in the extract.

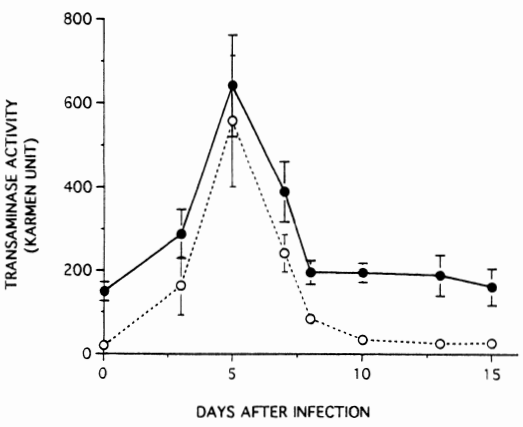

Fig. 1 Transaminase activities in sera from infected mice. BDF1 mice were infected with 1×10^4 embryonated eggs of *A. suum*. Transaminase activities are expressed as Karmen units. ●, glutamic-oxaloacetic transaminase (GOT); ○, glutamic-pyruvic transaminase (GPT); vertical bars, standard errors (SE).

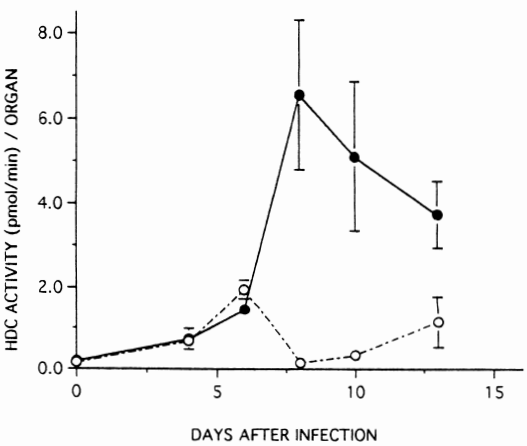

Fig. 2 L-histidine decarboxylase (HDC) activities in organs of infected mice. HDC activities are expressed as pmol/min/organ. ●, lung; ○, liver; vertical bars, SE.

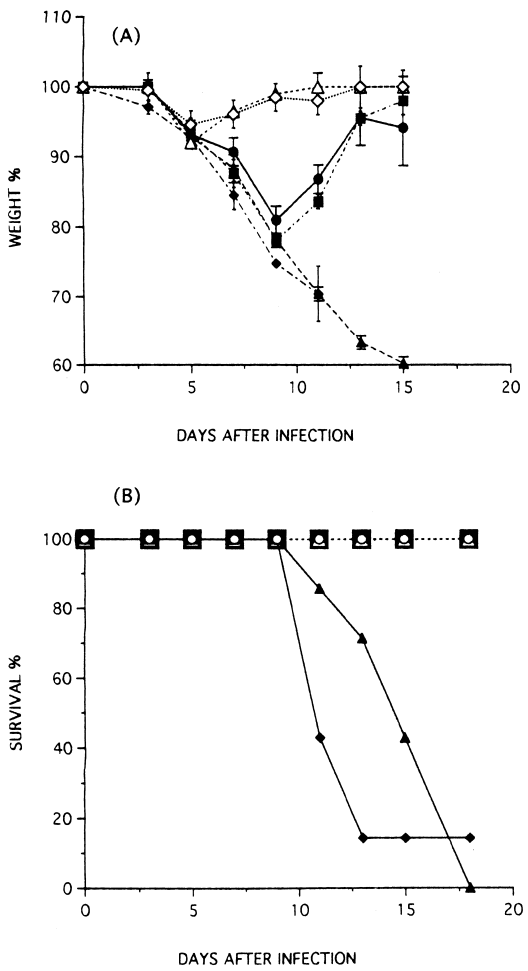

Fig. 3 Effects of a histamine H_2-receptor antagonist (famotidine, FA) on the recovery of infected mice. Normal mice or those infected with *A. suum* received two intraperitoneal injections (8 hours interval) of 0.1 ml of saline or saline containing 10, 30 or 100 μg of FA daily from the 3rd to the 9th day of infection. (A), changes in the weight of the mice. Data are expressed as percentages of the preinfection weight of the respective groups. ◇, normal mice given saline; △, normal mice given 30 μg of FA; ●, infected mice given saline; ■, infected mice given 10 μg of FA; ▲, infected mice given 30 μg of FA; ◆, infected mice given 100 μg of FA; vertical bars, SE. (B), Mice survival. Data are expressed as percentages of original number of respective group. ○, infected mice given saline; △, normal mice given 30 μg of FA; ■, infected mice given 10 μg of FA; ▲, infected mice given 30 μg of FA; ◆, infected mice given 100 μg of FA; vertical bars, SE.

Effects of a histamine H_2-receptor antagonist (famotidine)

The physiological significance of the increase in HDC activity was tested using the H_2-receptor antagonist, famotidine (FA). Four groups of mice (7 mice/group) were infected with 1×10^4 embryonated eggs of *A. suum*. Each group received two intraperitoneal injections per day (8 hour intervals) of 0.1 ml of saline or saline containing 10, 30 or 100 μg of FA from the 3rd to the 9th day of infection. One group of normal mice received injections of 30 μg FA. As seen in Fig. 3A, the infected mice lost body weight from the 5th day of infection. The loss of weight of the saline group of infected mice was maximal on the 9th day (about 20%). On the 13th day, the loss was almost recovered. The weight of the group of infected mice given 10 μg of FA was similar to that of the saline group. However, weight loss in the infected mice given 30 or 100 μg of FA never recovered and both groups began to die from the 11th day (Fig. 3B). FA itself (at least a series of 30 μg injections, total dose, 420 μg) had no effect on uninfected mice.

Corticosterone in plasma

The corticosterone concentrations in plasma of mice that were infected with 1×10^4 embryonated eggs of *A. suum* were determined by radioimmunoassay. Data are expressed as means and standard errors of four samples (Fig. 4). The corticosterone concentration in normal plasma was 155±14.8 ng/ml. In plasma from infected mice, that increased from the 3rd day with at least 2 peaks. The concentration at the first peak on the 3rd day was 226.5±32.2 ng/ml ($p<0.1$) and that at the second peak on the 7th day was 351.25±50.2 ng/ml ($p<0.01$). Therefore, the corticosterone level in the circulation was maximally elevated about 2.3-fold by the infection. The kinetics resembled that of IL-6 that was shown in our separate report (Komatsu, *et al.*, 1996). The correlation coefficient (R^2) between the corticosterone and IL-6 concentrations was 0.827.

Catecholamines in plasma

The kinetics of adrenalin, noradrenalin and dopamine concentrations in plasma from infected mice are shown in Fig. 5. The respective normal levels were 28.33±6.68, 25.63±4.84 and 3.64±0.87

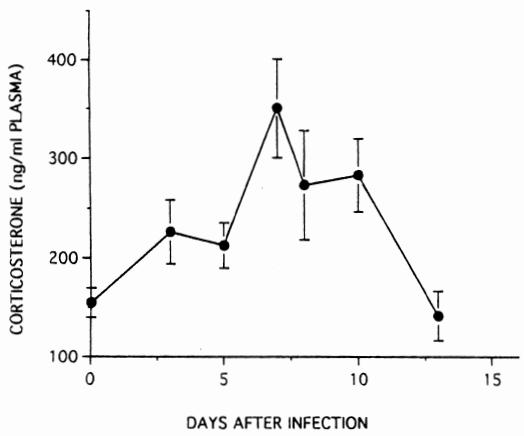

Fig. 4 Increase in corticosterone concentration in plasma of infected mice. The concentration is expressed as ng/ml plasma. Vertical bars, SE.

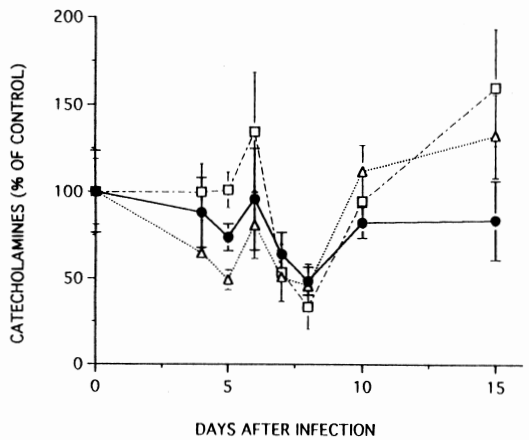

Fig. 5 Change in the concentrations of plasma catecholamines in infected mice. Data are expressed as percentages of preinfection levels. The preinfection levels of adrenalin, noradrenalin and dopamine are 28.33±6.68, 25.63±4.84 and 3.64±0.87 pmol/ml plasma, respectively. ●, adrenalin; △, noradrenalin; □, dopamine; vertical bars, SE.

pmol/ml. Data are expressed as percentages of normal levels (means and standard errors of 6 plasma). Adrenalin and noradrenalin concentrations decreased on the 5th (73.91±7.71%, 49.30±5.84%), 7th (64.01±12.65%, 50.70±14.04%) and 8th days (48.51±8.05%, 45.69±12.90%) of infection. The dopamine concentration decreased on the 7th (53.43±16.44%) and 8th days (33.50±13.13%). The decreases in adrenalin, noradrenalin and dopamine levels on 8th day were significant at $p<0.02$, $p<0.05$ and $p<0.01$, respectively.

Discussion

Liver injuries caused by larval migration are not usually evident upon external observation. However, changes in all the examined parameters seemed to be triggered at the time of larval migration into the liver. The incidence of liver damage caused by the migration was estimated by measuring transaminase activities in the circulation. Both GOT and GPT dramatically increased on the 5th day of infection (Fig. 1). The remarkable increases in both enzymatic activities were transient and decreased thereafter. The kinetics is coincident with that of larval migration into the liver that has been reported in our separate manuscript (Komatsu *et al.*, 1996). This indicates that the liver is transiently, but definitely injured by the larvae.

The activity of a histamine-producing enzyme, HDC, increased in target organs (Fig. 2). The increase in the lung was most remarkable. The rather low t value of Student's *t*-test ($p<0.1$) at the peak was due to the high diversity among elevated levels of the activity (4.534 to 10.055 pmol/organ/min). The *Ascaris* helminth contains various amines (Phillips *et al.*, 1975; Mishra *et al.*, 1984) and aromatic L-amino acid decarboxylase (Chaudhuri *et al.*, 1988). However, HDC in mammalian tissue requires pyridoxal-5'-phosphate as a coenzyme for histamine synthesis (Kahlson and Rosengren, 1968). The HDC activities shown in this report were estimated according to the assay method for this. By the method, no activities were detected in the extract of 1×10^5 embryonated eggs. Moreover, levels of the activity are quite different between organs that contain the almost same number of larvae. These confirm that the enzymatic activities are those of endogenous HDC in murine organs.

Various cell types besides mast cells have HDC and produce histamine (Kahlson and Rosengren, 1968; El-Ackad and Brody, 1975; Håkanson *et al.*, 1976; Kawaguchi-Nagata *et al.*, 1988; Aoi *et al.*, 1989). Histamine has a variety of physiological functions (Beaven, 1978). The histamine produc-

tion is enhanced in the wounded tissue. It plays a role in the repair of wounds by causing rapid tissue growth (Kahlson and Rosengren, 1968; Yamatodani *et al.*, 1983). When HDC is inhibited, the rate of healing of wound is retarded (Kahlson *et al.*, 1960) and the collagen formation is disordered (Sandberg, 1964). The nascent rather than liberated type of histamine acts in the repair. The tissue producing nascent histamine is characterized by high histamine-forming capacity, low histamine content and low capacity to bind histamine (Kahlson, 1962). In general, wound tissues have a high histamine-forming capacity, but a high turnover rate of it. As a result, the histamine content of these tissues is very low compared with normal tissue (Kahlson and Rosengren, 1968). We observed the same phenomenon. The histamine content of the liver decreased to 56.2±10.2% on the 5th day and that of the lung decreased to 65.1±4.6% of the normal level on the 7th day of infection (Data not shown in text). Therefore, our data indicate the operation of reparative reaction to the damage in target organs.

We have shown that the histamine H_2-receptor is involved in an important function for the survival of animals under larval helminth infection (Fig. 3). Famotidine (FA) is an antagonist of the histamine H_2-receptor with about a hundred times higher affinity with the receptor and longer retention time than cimetidine. The intraperitoneal injection of a total dose of 420 μg of FA (daily 60 μg for 7 days) had no effect on normal mice. The same dose of FA, however, induced a lethal effect on infected mice. The administration of a much higher dose of cimetidine elicited the same effect (Data not shown). The mechanism by which the blockade of the H_2-receptor leads to the mortality of infected mice remains unclear. It can not be excluded that the blockade of H_2-receptor interferes with repair reactions.

The administration of H_2-receptor antagonist may interfere with pulmonary function. Both H_1 and H_2-receptors are expressed on airway smooth muscle and the pulmonary vasculature of various mammals (Akpako, 1972; Tucker *et al.*, 1973; Woods *et al.*, 1977; Barer *et al.*, 1978; Ahmed and King, 1986). The administration of the H_2-receptor antagonist results in selective stimulation of H_1-receptor and the reverse is also true. In the pulmonary vasculature, the stimulation of H_1-receptors causes vasoconstriction, whereas that of H_2-receptors results in the vasodilatation (Akpako, 1972; Tucker *et al.*, 1973; Woods *et al.*, 1977; Barer *et al.*, 1978). The stimulation of receptors is usually mediated by liberated rather than nascent histamine. Although the histamine content in the circulation was not determined in this experiment, it has been documented that the histamine forming capacity in the whole body is elevated by wounding local tissues (Kahlson and Rosengren, 1968). Therefore, the death induced by H_2-receptor antagonist may be due to the hypoxia, resulting in the additional stress to the infected mice.

The increase in corticosterone in the circulation (Fig. 4) indicates the activation of the hypothalamo-pituitary-adrenal (HPA) axis. IL-6 is an important factor that mediates the activation of the HPA axis. IL-6 stimulates the releases of corticotropin-releasing factor and adrenocorticotropic hormone (ACTH), and can act synergistically on the ACTH-stimulated release of corticosterone (Lyson *et al.*, 1991; Naitoh *et al.*, 1988; Salas *et al.*, 1990). In the circulation of infected mice, the kinetics of corticosterone almost parallels that of IL-6 (Komatsu *et al.*, 1996). The correlation coefficient (R^2) between them was 0.827. On the other hand, IFN-α inhibits HPA secretion following peripheral and central administration (Saphier *et al.*, 1994). Although we did not determine the type of IFN elevated in the infected mice, it may not be relevant to the secretion of corticosterone.

The secretion of corticosterone caused by LPS is mediated by histamine produced through the induction of histidine decarboxylase in non-mast cells (Suzuki and Nakao, 1986). In addition, endogenous histamine and PGE_2, which are released during carrageenan-induced acute inflammation, are responsible for the increase of corticosterone in both plasma and inflammatory exudate (Chio and Sin, 1992). Therefore, histamine may also relate to the secretion of corticosterone in the infected mice.

Both corticosterone and catecholamine-mediated mechanisms are operative in some stress-induced suppression of cellular immunity (Dobbs *et al.*, 1993). However, catecholamine does not seem to affect the immune system in the infected mice, because concentrations of all three catecholamines

in the circulation are significantly decreased (Fig. 5). Whether the decrease is due to the inhibition of secretion or the rapid catabolism was not tested here. It is noted that various stressors such as footshock, restraint or virus infection activate the catabolism of catecholamines at least in cerebral tissues (Dunn, 1988a; Dunn, 1988b).

In summary, larval *A. suum* infection is a complex of stimuli to induce various physiological reactions. The antigenic materials from larvae induce the B cell proliferation. On the other hand, some physiological reactions finally induce the corticosterone secretion and perturb the immune system.

Acknowledgments

We express our gratitude to Dr. K. Kondo (Department of Parasitology, Kanazawa University School of Medicine) for providing *A. suum* eggs.

References

1) Ahmed, T. and King, M. (1986): Suppression of pulmonary and systemic vascular histamine H_2-receptors in allergic sheep. J. Appl. Physiol., 60, 791–797.
2) Akpako, P. T. (1972): A dual action of histamine on guinea pig vessels. Br. J. Pharmacol., 44, 311–321.
3) Aoi, R., Nakashima, I., Kitamura, Y., Asai, H. and Nakano, K. (1989): Histamine synthesis by mouse T lymphocytes through induced histidine decarboxylase. Immunology, 66, 219–223.
4) Barer, G. R., Emery, C. J., Mohammad, F. H. and Munghall, I. P. F. (1978): H1- and H2-histamine receptor action on lung vessels: their relevance to hypoxia vasoconstriction. Q. J. Exp. Physiol. Cogn. Med. Sci., 63, 157–169.
5) Beaven, M. A. (1978): Histamine: Its role in physiological and pathological processes. Monogr. Allergy, 13, 1–89.
6) Blalock, J. E. (1989): A molecular basis for bidirectional communication between the immune and neuroendocrine systems. Physiol. Rev., 69, 1–32.
7) Chaudhuri, J., Martin, R. E. and Donahue, M. J. (1988): Tryptophan hydroxylase and aromatic L-amino acid decarboxylase activities in the tissues of adult *Ascaris suum*. Int. J. Parasitol., 18, 1–6.
8) Chio, S. L. and Sin, Y. M. (1992): Changes in corticosterone levels under different degrees of acute inflammation in mice. Agents Actions, 36, 93–98.
9) Crandall, C. A. and Crandall, R. B. (1971): *A. suum*: Immunoglobulin responses in mice. Exp. Parasitol., 30, 426–437.
10) Crandall, C. A. and Crandall, R. B. (1976): *Ascaris suum*: Immunosuppression in mice during acute infection. Exp. Parasitol., 40, 363–372.
11) Dobbs, C. M., Vasquez, M., Glaser, R. and Sheridan, J. F. (1993): Mechanisms of stress-induced modulation of viral pathogenesis and immunity. J. Neuroimmunol., 48, 151–160.
12) Dunn, A. J. (1988a): Stress-related changes in cerebral catecholamine and indoleamine metabolism: lack of effect of adrenalectomy and corticosterone. J. Neurochem., 51, 406–412.
13) Dunn, A. J. (1988b): Stress-related activation of cerebral dopaminergic systems. Ann. N. Y. Acad. Sci., 537, 188–205.
14) El-Ackad, T. M. and Brody, M. J. (1975): Fluorescence histochemical localization of non-mast cell histamine. In Neuropsychopharmacology, Boissier, J. R., Hippus, H. and Pichot, P., ed., Excerpta Medica, Amsterdam, 551–559.
15) Freire-Garabal, M., Belmonte, A., Orallo, F., Couceiro, J. and Núñez, M. J. (1991): Effects of alprazolam on T-cell immunosuppressive response to surgical stress in mice. Cancer Lett., 58, 183–187.
16) Goetzl, E. J., Adelman, D. C. and Sreedharan, S. P. (1990): Neuroimmunology. Adv. Immunol., 48, 161–190.
17) Håkanson, R., Larsson, L.-I., Liedberg, G. and Sundler, F. (1976): The histamine-storing enterochromaffin-like cells of the rat stomach. In Chromaffin, Enterochromaffin and Related Cells, Coupland, R. E. and Fujita, T., ed., Elsevier, Amsterdam, 243–263.
18) Kahlson, G., Nilsson, K., Rosengren, E. and Zederfeldt, B. (1960): Wound healing as dependent on rate of histamine formation. Lancet, 279, 230–234.
19) Kahlson, G. (1962): New approaches to the physiology of histamine. Perspectives Biol. Med., 5, 179–197.
20) Kahlson, G. and Rosengren, E. (1968): New approaches to the physiology of histamine. New Approaches to the Physiology of Histamine. Physiol. Rev., 48, 155–196.
21) Kawaguchi-Nagata, K., Watanabe, T., Yamatodani, A., Inoue, M., Asai, H., Tamura, T., Wada, H., Shoji, K. and Kitamura, Y. (1988): *In vitro* increase of histidine decarboxylase activity and release of histamine by peritoneal resident cells of mast cell-deficient W/W^v mice; possible involvement of macrophages. J. Biochem., 103, 24–30.
22) Komatsu, T., Yoshimoto, T., Okamura, H., Yamatodani, A., Saeki, N. and Shinka, S. (1996): Immunological parameters affected by larval *Ascaris suum* Infection. (in press).
23) Lyson, K., Milenkovic and McCann, S. M. (1991): The stimulatory effect of interleukin-6 on corticotropin-releasing factor and thyrotropin-releasing hormone secretion *in vitro*. Prog. Neuroendocrinol. Immunol., 4, 161–165.
24) Mishra, S. K., Sen, R. and Ghatak, S. (1984): Ascaris lumbricoides and Ascaridia galli: Biogenic amines in adults and developmental stages. Exp. Parasitol., 57, 34–39.
25) Mitchell, G. F., Hogarth-Scott, R. S., Edwards, R. D.,

Lewers, H. M., Cousins, G. and Moore, T. (1976): Studies on immune responses to parasite antigens in mice. I. Ascaris suum larvae numbers and antiphosphorylcholine responses in infected mice of various strains and in hypothymic nu/nu mice. Int. Arch. Allergy Appl. Immunol., 52, 64–78.

26) Munck, A., Guyre, P. M. and Holbrook, N. J. (1984): Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev., 5, 25–44.

27) Naitoh, Y., Fukata, J., Tominaga, T., Tamai, S., Mori, K. and Imura, H. (1988): Interleukin-6 stimulates the secretion of adrenocorticotropic hormone in conscious, freely-moving rats. Biochem. Biophys. Res. Commun., 155, 1459–1463.

28) Nohta, H., Mitsui, A. and Ohkura, Y. (1984): Spectrofluorimetric determination of catecholamines with 1,2-diphenylethylenediamine. Analytica Chimica Acta, 165, 171–176.

29) Phillips, J. L., Sturman, G. and West, G. B. (1975): The presence of histamine in the tissues of Ascaris suum. 1975. Gen. Pharmacol., 6, 295–297.

30) Salas, M. A., Evans, S. W., Levell, M. J. and Whicher, J. T. (1990): Interleukin-6 and ACTH act synergistically to stimulate the release of corticosterone from adrenal gland cells. Clin. Exp. Immunol., 79, 470–473.

31) Sandberg, N. (1964): Granulation tissue hydroxyproline in the rat after inhibition of histamine formation. Acta Chir. Scand., 127, 22–34.

32) Saphier, D., Roerig, S. C., Ito, C., Vlasak, W. R., Farrar, G. E., Broyles, J. E. and Welch, J. E. (1994): Inhibition of neural and neuroendocrine activity by alpha-interferon: neuroendocrine, electrophysiological, and biochemical studies in the rat. Brain Behav. Immun., 8, 37–56.

33) Sprent, J. F. A. (1952): On the migratory behaviour of the larvae of various ascaris species in white mice. I. Distribution of larvae in tissues. J. Infect. Dis., 90, 165–176.

34) Stein, M., Keller, S. E. and Schleifer, S. J. (1985): Stress and immunomodulation: The role of depression and neuroendocrine function. J. Immunol., 135, 827–833.

35) Suzuki, S. and Nakano, K. (1986): Possible role of endogenous histamine in mediation of LPS-induced secretion of corticosterone in mice. Biochem. Pharmacol., 35, 3039–3043.

36) Tucker, A., Weir, E. K., Reeves, J. T. and Grover, R. F. (1973): Histamine H1- and H2-receptors in pulmonary and systemic vasculature of the dog. Am. J. Physiol., 229, 1008–1013.

37) Woods, J. R., Jr., Brinkman, C. R., III, Dandavino, A., Murayama, K. and Assali, N. S. (1977): Action of histamine and H1 and H2 blockers on the cardiopulmonary circulation. Am. J. Physiol., 232 (Heart Circ. Physiol. 1), H73–H78.

38) Yamatodani, A., Watanabe, T. and Wada, H. (1983): Methods for determination of histamine in biological materials. In Methods in Biogenic Amine Research, Parvez, S., Nagatsu, T., Nagatsu, I. and Parvez, H., ed., Elsevier, Amsterdam, New York, Oxford, 663–687.

39) Yamatodani, A., Fukuda, H., Iwaeda, T., Watanabe, T. and Wada, H. (1985): High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: Cation-exchange chromatography coupled with post-column derivatization fluorometry. J. Chromatogr., 344, 115–123.