[Jap. J. Parasit., Vol. 28, No. 5, 339-346, 1979]

Effect of Neonatal Thymectomy and Administration of Immunosuppressants on the Acquired Resistance of Mice to Infection with the Nematode, *Trichuris muris*

MASANOBU TANABE*, YUKIO HOSAKA, YOICHI ITO†,
FUSAO SENDA‡ AND TOSHIRO OKUDA‡
Department of Parasitology, National Institute of Health Japan, Tokyo, Japan

(Received for publication; April 27, 1979)

Introduction

Following a primary infection with *Trichuris muris*, the majority of albino mice acquire a resistance which eliminates almost all parasites before maturity and prevents the establishment of subsequent challenge infection (Wakelin, 1967; Tanabe *et al.*, 1974). Campbell *et al.* (1968) found that the administration of a steroidal anti-inflamatory agent, cortisone, potently suppressed the acquired resistance of mice, whereas no suppressive effect was observed in mice treated with a non-steroidal anti-inflamatory agent, indomethacin. This finding suggests that the host immune response may be involved in the mechanism of the spontaneous expulsion of worms. Wakelin and his coworker (1975 1976), also studying on the acquired resistance of mice to *T. muris* infection, suggested that multiphasic immune processes involving antibody and lymphoid cell components participate in the immune expulsion of worms. However, the details on the host immune mechanism have not been informed.

Present communication deals with the effectiveness of immunosuppressive means, such as neonatal thymectomy, administration of antithymocyte serum, antilymphocyte serum or cortisone, on the acquired resistance to infection with *T. muris* in mice.

Materials and Methods

Parasite: The strain of *Trichuris muris*, which was kindly supplyed by Dr. E. H. Pike, has been maintained in our laboratory for about 10 years using gpc strain mice treated with cortisone (Nippon Merck Banyu, Ltd, Tokyo, Japan). Egg cultivation and infection were performed as follows. The eggs in feces were collected by floating method with zinc sulfate solution. Embryonated eggs were obtained by egg cultivation in 0.5% formalin at 28 C for 9 weeks. Over 90% of eggs were embryonated. The eggs were washed several times with tap water and suspended in 1% tragacanth gum (Wako Junyaku, Ltd, Tokyo, Japan) solution. One half ml of the egg suspension were inoculated into mice orally using a gastric catheter attached to a syringe.

Animals: White rabbits weighing about 3 kg were used for the preparation of antisera. Male and female gpc strain mice were

* *Department of Parasitology, School of Medicine Keio University, Shinanomachi 35, Shinjuku-ku Tokyo 160, Japan.*
† *Department of Parasitology, School of Medicine Kitasato University, Asamizodai 1, Sagamihara-shi Kanagawa-ken 228, Japan.*
‡ *Department of Parasitology, Kyorin College School of Medical Technology, 6-20-2 Shinkawa, Mitaka-shi Tokyo Tokyo 181, Japan*

used throughout the experiments. For *thymectomy* experiment, new born mice were obtained from bleeding pairs in our laboratory. All mice were fed ordinary mouse pellets and water *ad libitum*. Mice were initially exposed to infection with 300–400 embryonated eggs of *T. muris* at 5 weeks of age. Challenge infection was performed on day 35 of initial infection.

The mice were dissected on day 35 of initial infection, and the intestines were removed, slit and washed in cold physiological saline. Number of worms were counted under a dissecting microscope. In the case of challenge infection, mice were killed on day 8 of challenge infection. The intestines were treated in the same manner as above, and number of larvae were counted under a light microscope.

Neonatal and post-neonatal thymectomy: New born mice were thymectomized within 16 hours after birth according to the method described by Okamoto (1968). Approximately two thirds of the newborns in each litter were neonatally thymectomized, and remainders served as sham-operated control. Post-neonatal thymectomy was performed on 7 days after birth. Thymectomized and sham-operated mice were marked and returned to their mother until weaned. Thereafter all were fed ordinary mouse pellets and water *ad libitum*.

Total post-operative mortality of neonatally thymectomized mice due to surgery and maternal neglect or cannibalisation approached about 50%. Approximately 25% of the surviving animals were lost as a result of runt disease thereafter. Two animals surviving during the experimental periods were found on examination to have some residual thymus. Data obtained from these two mice were omitted.

Preparation of antisera: Two kinds of antisera were prepared to compare their suppressive effect. One was rabbit antiserum against thymocytes and another was rabbit antiserum against lymphocytes from the mesenteric lymph node of gpc strain mice.

Antithymocyte serum (ATS) was prepared as follows. Whole thymus glands of 2-week-old mice were washed in cold Eagle's MEM medium, cut into small pieces, and gently triturated with glass homogenizer. Viable cell suspension was obtained by filtration of homogenate through a sieve and washed three times in cold MEM medium. White rabbits received two intravenous injections of 3×10^8 viable thymocytes at two-week interval, and were bled 1 week after the last immunization. The blood was allowed to clot at 37 C for 3 hours, and then sera were isolated by centrifugation. The sera were inactivated at 56 C for 30 min, absorbed with packed mouse erythrocytes and stored at −20 C until used.

Antilymphocyte serum (ALS) was prepared in the same manner as above using 2×10^8 cells from mesenteric lymph node of gpc strain mice. Normal rabbit serum (NRS) also inactivated and absorbed with mouse erythrocytes.

In order to examine the antibody activities in ATS and ALS against mouse serum proteins, immunoelectrophoretic analysis was performed according to the method described by Okamoto (1972). Fine precipitation lines were observed between rabbit antiserum against mouse serum (Hoechst Japan, Ltd, Tokyo, Japan) and normal or *T. muris* infected mouse sera, whereas no precipitation band was detected between mouse sera and ATS or ALS. This finding, therefore, indicated that ATS and ALS used in this study had no influence on mouse serum proteins.

Before starting the experiments, the leuko-agglutination titre of antisera was determined according to the method described by Grey *et al.* (1966) using thymocytes or mesenteric lymph node cells from gpc strain mice as a target cell. As shown in Table 1, thymocyte agglutination titers of ATS and ALS were equally 1 : 256. Lymphocyte agglutination titres of ATS and ALS were 1 : 256 and 1 : 128 respectively. NRS did not reveal its agglutination activity.

Table 1 The leukoagglutination titer of NRS, ALS andA TS against thymocytes or mesenteric lymph node cells

Antiserum	Target cell	
	Thymotyte	Mesenteric lymph node cell
NRS	Nil	Nil
ALS	1 : 256	1 : 128
ATS	1 : 256	1 : 256

The leukoagglutination titer was expressed as the reciprocal values of serum dilution.

Results

Experiment I: This experiment was designed to determine whether neonatal and post-neonatal thymectomy reveal their suppressive effect on the acquired resistance of gpc strain mice to initial infection with *Trichuris muris*. As shown in Table 2, there was significant difference between mean number of worms recovered from neonatally thymectomized mice and that from sham-operated controls, but no significant difference was observed between post-neonatally thymectomized and sham-operated mice. Furthermore, almost all neonatally thymectomized mice harboured a large number of adult worms, whereas the majority of sham-operated and post-neonatally thymectomized mice were free from parasites. Only one female mice thymectomized at 7 days old was positive for parasites. In this case, only 5 parasites were recovered. These evidences indicate that neonatal thymectomy closely associates with impairments of the ability to induce the resistance to initial infection with *T. muris*.

Experiment II: This experiment was designed to determine whether ATS, ALS or cortisone administration during early phase of initial infection reveals their suppressive effect on the acquired resistance to initial infection with *T. muris*. The results were summarized in Table 3. All mice of Group I (non-treated control) showed a striking resistance; they were all free from parasite. Repeated injections of NRS (Group II) failed to suppress the worm expulsion, whereas ATS or cortisone administration significantly suppressed the development of the acquired resistance of mice. A large number of worms were recovered from almost all mice of these groups. In contrast, ALS administration revealed weak suppressive effect on the acquired resistance; a half of mice were free from parasite and mean number of worms recovered was markedly lower than those from ATS- or cortisone-treated groups.

Experiment III: This experiment was ar-

Table 2 Effect of neonatal and post-neonatal thymectomy on the acquired resistance of mice to initial infection with 300 embryonated eggs of *Trichuris muris*

Group	Sex	No. of mice examined	Infection* rate	No. of mice with following worm counts				Mean No. of worms recovered
				0	1–60	61–120	121<	
Thymectomy at birth	♂	13	12/13	1	3	4	5	130
	♀	12	12/12	0	4	4	4	127
Partial thymectomy at birth	♂	15	1/15	14	1	0	0	1
	♀	10	1/10	9	1	0	0	1
Thymectomy at 7 days old	♂	7	0/7	7	0	0	0	0
	♀	5	1/5	4	1	0	0	1

* Number of mice infected / number of mice examined
Male and female mice were exposed to infection at 5 weeks of age and dissected on day 35 of infection.

ranged to determine whether ATS, ALS or cortisone administration during early phase of initial infection reveals their suppressive effect on the acquired resistance of mice to challenge infection with *T. muris*. As shown in Table 4, almost all mice of Group I (resistant control) showed a striking acquired resistance and completely eliminated the larvae from challenge infection. In Group II (nonresistant control), however, all the experimental animals harboured a large number of parasites. Administration of NRS (Group III) failed to suppress the acquired resistance to challenge infection; almost all mice were negative for parasites. The interesting finding was obtained from the

Table 3 Effect of ATS, ALS or cortisone administration during early phase of initial infection on the acquired resistance of mice to initial infection with *Trichuris muris*

Group	Administration	No. of mice examined	Infection* rate	No. of mice with following worm counts				Mean No. of worms recovered
				0	1-200	201-400	400<	
I	—	5	0/5	5	0	0	0	0
II	NRS	9	2/9	7	2	0	0	11
III	ALS	19	10/19	9	8	2	0	40
IV	ATS	11	11/11	0	2	8	1	300
V	cortisone	9	7/9	2	3	4	0	169

* Number of mice infected / number of mice examined

Male mice were only used in this experiment. All mice were exposed to 400 embryonated eggs at 5 weeks of age and dissected on day 35 of infection. Five mice in Group I were nontreated control. Thirty-nine mice were divided into 3 groups and were injected intraperitoneally with 0.2 ml of NRS (Group II), ALS (Group III) and ATS (Group IV) on days-2, 0, 2, 4, 6, 8, 10, 12 and 14 of infection. Nine mice in Group V were injected intramuscularly with 2.5 mg cortisone per head on days 0, 1, 9 and 10 of infection.

Table 4 Effect of ATS, ALS or cortisone administration during early phase of initial infection on the acquired resistance of mice to challenge infection with *Trichuris muris*

Group	Administration	No. of mice examined	Infection* rate	No. of mice with following worm counts				Mean No. of worms recovered
				0	1-60	61-120	121<	
I	—	9	1/9	8	1	0	0	3
II	—	9	9/9	0	0	7	2	86
III	NRS	9	1/9	8	1	0	0	1
IV	ALS	9	2/9	7	2	0	0	1
V	ATS	8	8/8	0	1	7	0	68
VI	cortisone	9	9/9	0	2	6	1	77

* Number of mice infected / number of mice examined

Male mice were only used in this experiment. All mice except for Group II were initially exposed to 400 embryonated eggs at 5 weeks of age and reexposed to 250 eggs on day 35 of initial infection. Nine mice of Group II (nonresistant control) were only exposed to challenge infection. Details of the administration of NRS (Group III), ALS (Group IV), ATS (Group V) and cortisone (Group VI) were given in the legend to Table 3. Nine mice in Group I were resistant control. All mice were dissected on day 8 of challenge infection.

Table 5 Effect of ATS, ALS or cortisone administration during challenge infection on the acquired resistance of mice to challenge infection with *Trichuris muris*

Group	Administration	No. of mice examined	Infection* rate	No. of mice with following worm counts				Mean No. of worms recovered
				0	1-60	61-120	121<	
I	—	9	2/9	7	2	0	0	3
II	—	9	9/9	0	4	3	2	86
III	NRS	10	1/10	9	1	0	0	1
IV	ALS	11	6/11	5	6	0	0	2
V	ATS	10	9/10	1	6	3	0	34
VI	cortisone	7	7/7	0	7	0	0	31

* Number of mice infected / number of mice examined

Male mice were only used in this experiment. All mice except for Group II were initially exposed to 400 embryonated eggs at 5 weeks of age and reexposed to 250 eggs on day 35 of initial infection. Nine mice of Group II (nonresistant control) were only exposed to challenge infection. Thirty-one mice were divided into 3 groups and were injected intraperitoneally with 0.2 ml NRS (Group III), ALS (Group IV) and ATS (GroupV) on days-2, 0, 2, 4, and 6 of challenge infection. Seven mice in Group VI were injected intramuscularly with 2.5 mg cortisone per head on days -2, 0, 3 and 4 of challenge infection. Nine mice in Group I were resistant control. All mice were dissected on day 8 of challenge infection.

results of Group IV, V and VI. The vast majority of mice treated with ALS (Group IV) failed to suppress the acquired resistance, whereas all mice treated with ATS (Group V) or cortisone (Group VI) harboured a large number of larvae from challenge infection. Besides, mean number of worms recovered from Group V and VI were nearly equal to that of non-resistant control.

Experiment IV: This experiment was arranged to determine whether ATS, ALS or cortisone administration during challenge infection reveals their suppressive effect on the acquired resistance of mice to challenge infection with *T. muris*. The results were summarized in Table 5. All mice of Group I (resistant control) showed a striking resistance and completely eliminated the larvae from challenge infection. In Group II (nonresistant control), however, almost all mice were positive for larvae. Administration of NRS (Group III) failed to suppress the worm expulsion, whereas ATS or cortisone treatment markedly suppressed the acquired resistance developed during initial immunizing infection. The vast majority of mice in Group V and VI harboured many larvae from challenge infection. However, it appeared that number of worms recovered from these groups were lower than that from nonresistant mice (Group II). ALS treatment (Group IV) failed to suppress the worm expulsion from mice ; mean number of worms recovered was very low and was nearly equal to those from resistant mice (Group I) or NRS-treated mice (Group III).

Discussion

As shown in results, the acquired resistance of gpc strain mice to infection with *Trichuris muris* was potently depressed by the administration of antithymocyte serum or cortisone, and by neonatal thymectomy. The suppressive effect of these immunosuppressants on the acquired resistance has been observed in *Hymenolepis nana* (Okamoto, 1969, 1970, 1972) and in *Nippostrongylus brasiliensis* (Kelly, 1972). It is, in general, considered that neonatal thymectomy and the administration of ATS, ALS or cortisone are associated with a specific diminution in

the population of recirculating long-lived small lymphocytes playing a role in cell-mediated immunity or in as a helper T cell to antibody production (Dougherty *et al.*, 1964; Levey and Medawar, 1966; Miller and Osoba, 1967). It is, therefore, assumed that the acquired resistance of mice to *T. muris* infection is probably mediated by host immune response, particularly by T lymphocyte-dependent immune reaction.

The extensive studies on the self-cure reaction to *N. brasiliensis* showed that multiphasic immune processes involving antibody and lymphoid cell components participate in the immune expulsion of worms (Dineen *et al.*, 1973; Jones and Ogilvie, 1971; Keller and Keist, 1972). In this system, a local anaphylactic reaction produced by IgE-allergen interaction induced a severe histopathological change and a macromolecular leakage in the parasitized situs (Murray *et al.*, 1971). Some authors, therefore, suggested that such structural and physiological changes in the intestine play a major role in the immune expulsion of *N. brasiliensis* (Murray *et al.*, 1971; Hogarth-Scott and Bingley, 1971). The histopathological observation demonstrated a mild morphological change in the submucosa of the caecum just before and after the spontaneous expulsion of *T. muris* occurred; only a moderate edematous change and the infiltration of lymphocytes, eosinophils and plasma cells were observed (Tanabe, unpublished data). Ito and coworker (1977, 1979) detected PCA reactive antibody in sera of the resistant mice previously exposed to infection with *T. muris*, using a adult worm antigen, and observed that the worm expulsion from the mice was accelerated by the intraperitoneal immunization with the mixture of $Al(OH)_3$ gel and the antigen. It is, therefore, likely that a local anaphylactic reaction in the parasitized situs probably plays some role in immune expulsion of *T. muris* from the mice.

Selby and Wakelin (1973) investigated on the immune expulsion of *T. muris* from mice, and found that the acquired resistance could be conferred to normal recipient mice by sera or lymph node cells from the presensitized mice against *T. muris*. Immune serum, however, had no effect when the recipient mice had been immunosuppressed by sublethal or lethal irradiation (Wakelin, 1975; Wakelin and Selby, 1975). In contrast, immune lymph node cells could restore the worm expulsion of *T. muris* from the irradiated mice. They, therefore, suggested that as far as primary infection the sequential activities of antibody mediated and lymphoid cell-mediated components participate in the immune expulsion of this parasite from mice (Wakelin and Selby, 1976). Thus, the present study suggests that the depression of the acquired resistance of mice by the administration of immunosuppressants may result from their suppressive effects on both or either T cell-dependent antibody production and/or cell-mediated immunity.

Summary

Neonatal thymectomy markedly suppressed the development of the acquired resistance in gpc strain mice to initial infection with *Trichuris muris*. Thymectomy at 7 days old, however, failed to suppress the worm expulsion from mice.

Antithymocyte serum or cortisone administration during early phase of initial infection potently suppressed the development of the acquired resistance to both initial and challenge infections. Furthermore, administration of these immunosuppressants during challenge infection also suppressed the acquired resistance to challenge infection in the resistant mice previously exposed to initial immunizing infection. On the other hand, the leukoagglutination titre of ALS was nearly equal to that of ATS, but ALS had less suppressive effect on the acquired resistance than ATS.

These evidences indicate that host immune response, particularly T cell-dependent immune response, probably plays a major role in the immune expulsion of *T. muris* from gpc strain mice.

Acknowledgement

The authors wish to express their gratitude to Dr. Keizo Asami of the Department of Parasitology, Keio University for his helpful criticisms. The authors also wish to thank to Dr. Tatsushi Ishizaki of the Department of Internal Medicine, Dokkyo University, Dr. Kenichi Okamoto of the Department of Parasitology, Showa University and Dr. Masae Isoda of the Department of Pathology, Nippon Veterinary and Zootechnical College for their helpful suggestions and criticisms.

References

1) Campbell, W. C. (1968): Effect of anti-inflamatory agents on spontaneous cure of *Trichuris muris* and *Trichinella spiralis* in mice. J. Parasit., 54, 452-456.
2) Dineen, J. K., Ogilvie, B. M. and Kelly, J. D. (1973): Expulsion of *Nippostrongylus brasiliensis* from the intestine of rats: Collaboration between humoral and cellular components of the immune response. Immunol., 24, 467-475.
3) Dougherty, T. F., Berliner, M. L., Schneebeli, G. L. and Berliner, D. L. (1964); Hormonal control of lymphatic structure and function. Ann. N. Y. Acad. Sci., 113, 825-843.
4) Grey, J. G., Monaco, A. P., Wood, M. L. and Russell, P. S. (1966): Studies on heterologous anti-lymphocyte serum in mice. I. *In vitro* and *in vivo* properties. J. Immunol., 96, 217-228.
5) Hogarth-Scott, R. S. and Bingley, J. B. (1971): Prolongation and enhancement of *Nippostrongylus brasiliensis* infection in the laboratory rat by heterologous antiserum to rat peritoneal cells: A possible role for pharmacologically-active cells in immunity. Immunol., 21, 87-99.
6) Ito, Y. and Umiyama, H. (1977): Experimental studies on the infection of laboratory animals with *Trichuris muris* (4): Effect of the immunization with *T. muris* antigen. Jap. J. Parasit., 26 (Supplement), 23.
7) Ito, Y. and Saito, N. (1979): Experimental studies on the infection of laboratory animals with *Trichuris muris* (6): Effect of several doses of antigen on the expulsion of worms from C_{57}B1/6 mice. Jap. J. Parasit., 28 (Supplement), 55.
8) Jones, V. E. and Ogilvie, B. M. (1971): Protective immunity to *Nippostrongylus brasiliensis*: The sequence of events which expels worms from the rat intestine. Immunol., 20, 549-561.
9) Keller, R. and Keist, R. (1972): Protective immunity to *Nippostrongylus brasiliensis*: Central role of the lymphocyte in worm expulsion. Immunol., 22, 267-273.
10) Kelly, J. D. (1972): Immunological suppression of parasite rejection by removal of selected lymphocyte populations: The effect of neonatal thymectomy and anti-thymocyte serum administration on the resistance of rats to infection with the nematode, *Nippostrongylus brasiliensis*. Aust. J. Exp. Biol. Sci., 50, 477-489.
11) Levey, R. H. and Medawar, P. B. (1966): Nature and mode of action of anti-lymphocyte antiserum. Proc. Nat. Acad. Sci., 56, 1130-1137.
12) Miller, J. F. A. P. and Osoba, D. (1967): Current concepts of the immunological function of the thymus. Physiol. Rev., 47, 437-520.
13) Murray, M., Jarrett, W. F. H. and Jennings, F. W. (1971): Mast cells and macromolecular leak in intestinal immunological reactions: The influence of sex of rats infected with *Nippostrongplus brasiliensis*. Immunol., 21, 17-31.
14) Okamoto, K. (1968): Effect of neonatal thymectomy on acquired resistance to *Hymenolepis nana* in mice. Jap. J. Parasit., 17, 53-59.
15) Okamoto, K. (1969): Effect of cortisone on acquired resistance to *Hymenolepis nana* in mice. Jap. J. Parasit., 18, 591-594.
16) Okamoto, K. (1970); *Hymenolepis nana*: Depression and restoration of acquired immunity in neonatally thymectomized mice. Exp. Parasit., 27, 28-32.
17) Okamoto, K. (1972): *Hymenolepis nana*: Effect of antithymocyte serum on acquired immunity in mice. Exp. Parasit., 32, 56-61.
18) Selby, G. R. and Wakelin, D. (1973): Transfer of immunity against *Trichuris muris* in the mouse by serum and cells. Intereat., J. Parasit., 3, 717-722.
19) Tanabe, M., Hosaka, Y,. Ito, Y., Isoda, M., Senda, F. and Okuda, T. (1974): Studies on the acquired resistance of mice to *Trichuris muris* infection. Jap. J. Parasit., 23 (Supplement), 75.
20) Wakelin, D. (1967): Acquired immunity to *Trichuris muris* in the albino laboratory

mouse. Parasitol., 57, 515-524.
21) Wakelin, D. (1975): Immune expulsion of *Trichuris muris* from mice during a primary infection: Analysis of the components involved. Parasitol., 70, 397-405.
22) Wakelin, D. and Selby, G. R. (1976): Immune expulsion of *Trichuris muris* from resistant mice: Suppression by irradiation and restoration by transfer of lymphoid cells. Parasitol., 72, 41-50.

Trichuris muris 感染によりマウスに獲得される感染抵抗性に対する新生時胸腺剔出および免疫抑制剤の効果

田辺将信　保阪幸男　伊藤洋一　仙田房雄　奥田俊郎
（国立予防衛生研究所寄生虫部）

Albino マウスは *Trichuris muris* 感染により強い感染抵抗性を獲得し，虫体が成熟する以前にその大部分を排出する．さらに抵抗性獲得マウスは再感染に対しても強い抵抗性を発揮する．この抵抗性発現機構に宿主の免疫反応が関与することはすでに明らかとなっているが，その宿主免疫機構の詳細は未だ明らかではない．そこで我々はマウスを各種免疫抑制法で処置した場合の *T. muris* に対する感染抵抗性の変化を，虫体の排出の有無および回収虫体数を示標として観察し，以下の成績を得た．

（1）新生時胸腺剔出はマウスの感染抵抗性発現を著しく抑制した．しかし出生後7日目に胸腺剔出をした場合には，此の様な効果は観察されなかつた．

（2）初感染時に抗マウス胸腺細胞血清（ATS）あるいはコーチゾンで処置したマウスは，その初感染及び再感染に対する感染抵抗性に著しい抑制が認められた．さらにすでに抵抗性を獲得したマウスの再感染時に処置した場合にも，再感染に対する抵抗性発現をある程度抑制することができた．

（3）抗マウスリンパ球血清（ALS）は，ATS とほぼ同程度のリンパ球凝集活性を示したが，その抵抗性に対する抑制効果は ATS に比較し，はるかに弱かつた．

以上の成績から，*T. muris* 感染によりマウスに獲得される感染抵抗性には宿主のTリンパ球が主要な役割を演じる宿主免疫反応が関与しているものと推測された．