Oncospheres of *Taenia solium* and *T. saginata asiatica* develop into metacestodes in normal and immunosuppressed mice

I.C. Wang[1], Y.X. Ma[4], J.X. Guo[5], W.C. Chung[2], S.C. Lu[3], A. Ito[6] and P.C. Fan*

[1]Institute and Department of Parasitology, National Yangming University, [2]Department of Parasitology, Taipei Medical College, [3]Department of Parasitology, National Taiwan University, Taipei, Taiwan, Republic of China: [4]Henan Provincial Health and Antiendemic Station, [5]Zhengzhou King-Shui Hospital, Zhengzhou, Henan, Peoples' Republic of China: [6]Department of Parasitology, Gifu University School of Medicine, Gifu, Japan

Abstract

Normal and immunosuppressed mice were infected with oncospheres of *Taenia saginata asiatica* and *T. solium*. Although normal ICR mice were not susceptible to these two parasites, cysticerci were recovered from the immunosuppressed ones following venous injection. For *T. s. asiatica*, immunosuppressed ICR mice had an infection rate of 12.5% and six cysticerci of this parasite were recovered from three males. After injection of *T. solium* oncospheres, a high infection rate of 57% was obtained and 23 cysticerci were collected from 13 male immunosuppressed ICR mice. The immunosuppressed C57 mice had the highest infection rate (100%) and cysticercus recovery rate (2.4%) for *T. solium*. The infection rate and cysticercus recovery rate in six normal C57 mice were 40% and 3% respectively. The immunosuppressed ICR, Balb/c and C3H mice were also susceptible to *T. s. asiatica*.

Introduction

In 1985, Machnicka & Smyth described the very early post-oncospheral development of *Taenia saginata* in immunosuppressed mice. Recently, oncospheres of *T. s. asiatica* and *T. solium* were found to develop into cysticerci in severe combined immunodeficiency (SCID) mice (Ito *et al.*, 1997a,b). Moreover, the mature cysticerci in the subcutaneous tissues of the SCID mice remained viable 5 months after infection. Although the model of SCID mice is valuable in the study of human taeniid cestodes, these mice are expensive and are difficult to maintain in laboratories with insufficient equipment. Therefore, models of normal or immunosuppressed mice may be favourable alternatives. In the present study, we determined whether it is possible to employ normal or immunosuppressed mice as intermediate hosts of *T. s. asiatica* and *T. solium*.

*Author for correspondence.
Fax: 886 2 821 4670
E-mail: pcfan@ym.edu.tw

Materials and methods

Parasites and mice

Three adult worms of *T. solium* were collected from three patients with cysticercosis and taeniasis solium in King-Shui Hospital, Zhengzhou City, Henan Province, Mainland China, after chemotherapy with a mixture of areca and pumpkin seeds. These worms were intact but

https://doi.org/10.1017/S0022149X0070040X Published online by Cambridge University Press

without a scolex. They were sent immediately to our laboratory in Taipei by air mail.

Adult worms of *T. s. asiatica* were collected from infected aborigines at the mountainous areas of Tatung District of Ilan County, Lanyu District of Taitung County and Chienshih District of Hsinchu County in Taiwan, Republic of China.

Normal mice of ICR, Balb/cAnN, C3H/HeN and C57BL/6N strains were employed in the present study. They were purchased from Animal Centre, National Yangming University, Taipei, Taiwan. In the experimental mice, each normal mouse was treated with prednisolone ($20\,mg\,kg^{-1}$) or dexamethasone ($3\,mg\,kg^{-1}$) daily by oral administration or triamcinolone acetonide ($2.5\,mg\,kg^{-1}$) every 10 days by venous injection.

In vitro *hatching of oncospheres*

Eggs of *T. solium* and *T. s. asiatica* were collected from the last ten gravid proglottids of the tapeworm and kept in a refrigerator at 4°C. These eggs were hatched by the enzyme method of Stevenson (1983) with our modifications (Wang *et al*., 1997).

Experimental infections

In the susceptibility experiments, 500, 1000 or 5000 oncospheres of *T. solium* or *T. s. asiatica* were inoculated into the mice by subcutaneous or venous injection but the eggs by stomach tube. The mice were kept separately in an air-conditioned room and fed with standard food and drinking water.

Necropsy of mice

All mice were killed by ether overdose at various intervals after infection. They were then examined carefully and the number and locality of cysticerci recovered were recorded. The cyst recovery rate was calculated by the formula: No. of cysts recovered/(No. of mice in group × No. of eggs inoculated).

Results

Susceptibility of immunosuppressed male ICR mice to T. s. asiatica *and* T. solium *oncospheres*

The infection rates in mice administered orally with prednisolone were 20% for *T. s. asiatica* and 75% for *T. solium* (table 1). In the dexamethasone intravenous injection, mice were not susceptible to *T. s. asiatica* but had an infection rate of 40% for *T. solium*. After injection of triamcinolone acetonide, mice were susceptible to *T. s. asiatica* and *T. solium* with infection rates of 14% and 57% respectively. However, the cyst recovery rates in all mice were low (table 1).

Susceptibility of normal and immunosuppressed mice to T. solium *oncospheres*

Non-immunosuppressed ICR, Balb/c, and C3H mice were not susceptible to oncospheres of *T. solium*. ICR mice with a subcutaneous injection of triamcinolone acetonide were also not susceptible. However, the immunosuppressed Balb/c, C3H, and C57 mice were susceptible to the oncospheres with infection rates of 50%, 60% and 100% respectively. Moreover, the normal C57 mice were also found to have a high infection rate of 80% (table 2).

Susceptibility of immunosuppressed ICR mice

Immunosuppressed ICR mice treated with triamcinolone acetonide ($2.5\,mg\,kg^{-1}$ every 10 days) were infected with *T. solium*. The infection rate of female mice (80% or 4/5) was higher than that in males (50% or 2/4). In addition, the cysticercus recovery rate was also higher in females (0.26%) compared with that in males (0.05%).

Discussion

Recently, Ito *et al*. (1997a) succeeded in establishing a SCID mouse model for the development of cysticerci of *T. solium* and *T. s. asiatica*. This model has an advantage in that the cysticerci of these two species remained viable 5 months after experimental infection. However, the maintenance of this animal model is laborious, expensive and requires special equipment. In the present study, we have demonstrated that oncospheres of *T. solium* and *T. s. asiatica* can develop to cysticerci in immunosuppressed (ICR, Balb/c and C3H) mice and even in normal (C57) mice. We also found that C57 mice are the most suitable laboratory intermediate hosts for *T. solium* and may be also for *T. s. asiatica* when immunosuppressed, since they have a high infection rate and

Table 1. Susceptibility and cyst recovery of *Taenia solium* (*T.s.*) and *Taenia saginata asiatica* (*T.s.a.*) oncospheres in immunosuppressed male ICR mice following venous injection.

Taenia species	Administration method/immunosuppression agent	No. of mice	Cyst recovery	
		Killed/infected (%)	No.	%
T.s.a.	Oral, prednisolone 20 mg/kg/day	5/1(20)	1	0.04
T.s.	Oral, prednisolone 20 mg/kg/day	4/3(75)	4(1, 1, 2)	0.2
T.s.a.	Injection, dexamethasone 3 mg/kg/day	5/0(0)	0	0
T.s.	Injection, dexamethasone 3 mg/kg/day	5/2(40)	2(1, 1)	0.08
T.s.a.	Injection, triamcinolone acetonide 2.5 mg/kg/10 days	14/2(14)	5(4, 1)	0.07
T.s.	Injection, triamcinolone acetonide 2.5 mg/kg/10 days	14/8(57)	17(1, 1, 1, 1, 2, 2, 4, 5)	0.24

Five or 15 male ICR mice were each infected by tail intravenous injection with 500 oncospheres and all mice were killed 46 days after infection.

https://doi.org/10.1017/S0022149X0070040X Published online by Cambridge University Press

Table 2. Susceptibility and cyst recovery of *Taenia solium* oncospheres in normal and immunosuppressed female mice following subcutaneous injection with or without triamcinolone acetonide (2.5 mg kg^{-1} every 10 days).

	Triamcinolone			No. of mice	Cyst recovery	
Mouse strain	With	Without	Days of infection	Killed/infected (%)	No.	%
ICR	v		67	4/0(0)	0	0
ICR		v	67	5/0(0)	0	0
Balb/c	v		67	4/2(50)	17(15, 2)	0.43
Balb/c		v	67	5/0(0)	0	0
C3H	v		68	5/3(60)	6(3, 2, 1)	0.12
C3H		v	68	5/0(0)	0	0
C57	v		68	5/5(100)	120(38, 24, 22, 21, 15)	2.40
C57		v	68	5/1(20)	10	0.20
C57		v	109	5/1(20)	1	0.02
C57*		v	46	5/4(80)	9(4, 2, 2, 1)	0.04

Five mice were each infected with 1000 oncospheres except for one group (*) in which each mouse was inoculated with 5000 eggs by stomach tube.

cysticercus recovery rate. The immunosuppressed C57 mice are also suitable intermediate hosts as all cysticerci show growth and survival following transfer from other rodents (Wang *et al*., unpublished data). Moreover, in the establishment and maintenance of *T. solium* or *T. s. asiatica* in immunosuppressed or normal mice, the procedures are easy and labour-saving and the materials and equipment are inexpensive.

The choice of immunosuppressive drug is an important factor in the successful establishment of the mouse model for *T. solium* and *T. s. asiatica*. Hydrocortisone, methyl prednisolone and prednisolone tertiary butylacetate have been reported to be useful in studies on the development of various *Taenia* species in rodents (Kitaoka *et al*., 1990; Allan *et al*., 1991; Kamiya *et al*., 1991). Although the three drugs were all effective, triamcinolone acetonide is the preferred drug, since it is a long-term immunosuppressive drug and requires administration only for every 10 days. The other two are short-term drugs and require daily administration.

Although Yang *et al*. (1994) first reported a mouse model for *Cysticercus cellulosae*, our study is the first report of establishing a model for metacestodes of *T. s. asiatica* in normal and immunosuppressed mice. Large *C. cellulosae* were found in normal C57 mice with a high infection rate of 80%. Although *T. s. asiatica* cysticerci degenerated or calcified 1 or 2 months post-infection in pigs, they were all found to be viable for more than 7 months post-infection in mice, which is a longer period of time than reported by Ito *et al*. (1997a,b). It has been suggested that the size of the cysticercus in the intermediate mammalian host might be controlled by some immune response which cannot kill the established larvae (Mitchell *et al*., 1977; Lucas *et al*., 1980; Ito, 1985; Ishiwata *et al*., 1992; Dixon & Jenkins, 1995). Moreover, the successful development and long-term survival of cysticerci in mice may also be related to the immune response. However, further studies are required to confirm these suggestions.

Nevertheless, we have successfully established mouse models for the cysticerci of *T. solium* and *T. s. asiatica*. In these new models, the maintenance of the host animals requires no special equipment, is inexpensive and less laborious.

Acknowledgements

The authors wish to express their sincere thanks to the National Science Council, Republic of China, for support of this research grant (NSC84-2332-B010-076), and to Mr C.Y. Lin, Miss P. Huang and Miss. C.W. Yen for their technical assistance.

References

Allan, J.C., Garcia-Dominguez, C., Craig, P.S., Rogan, M.T., Lowe, B.S. & Flisser, A. (1991) Sexual development of *Taenia solium* in hamsters. *Annals of Tropical Medicine and Parasitology* **85,** 573–576.

Dixon, J.B. & Jenkins, P. (1995) Immunology of mammalian metacestode infections. I. Antigens, protective immunity and immunopathology. *Helminthological Abstracts* **64,** 533–542.

Fan, P.C., Lin, C.Y., Chen, C.C. & Chung, W.C. (1995) Morphological description of *Taenia saginata asiatica* (Cyclophyllidea: Taeniidae) from man in Asia. *Journal of Helminthology* **69,** 299–303.

Ishiwata, K., Oku, Y., Ito, M. & Kamiya, M. (1992) Responses to larval *Taenia taeniaeformis* in mice with severe combined immunodeficiency (scid). *Parasitology* **104,** 363–369.

Ito, A. (1985) Thymus dependency of induced immune responses against *Hymenolepis nana* (cestode) using congenitally athymic nude mice. *Clinical and Experimental Immunology* **60,** 87–94.

Ito, A., Chung, W.C., Chen, C.C., Ito, M., Endo, S., Okamoto, M. & Fan, P.C. (1997a) Human *Taenia* eggs develop into cysticerci in *scid* mice. *Parasitology* **114,** 85–88.

Ito, A., Ito, M., Eom, K.S., Chung, W.C., Chen, C.C., Ma, L., Endo, S. & Fan, P.C. (1997b) *In vitro* hatched oncospheres of Asian *Taenia* from Korea and Taiwan develop into cysticerci in the peritoneal cavity of female *scid* (severe combined immunodeficiency) mice. *International Journal for Parasitology* **27,** 631–633.

Kamiya, M., Sato, H., Kitaoka, M., Ishiwata, K., Oku, Y., Ito, M. & Gathura, P. (1991) Laboratory rodent models for the tapeworm-stage of *Taenia saginata* and other

https://doi.org/10.1017/S0022149X0070040X Published online by Cambridge University Press

related taeniid species. *Southeast Asian Journal of Tropical Medicine and Public Health* **22** (Supplement), 262–267.

Kitaoka, K., Oku, Y., Okamoto, M. & Kamiya, M. (1990) Development and sexual maturation of *Taenia crassiceps* (Cestoda) in the golden hamster. *Journal of Parasitology* **76,** 399–402.

Lucas, S.B., Hassounah, O.A., Muller, R. & Doenhoff, M. (1980) Abnormal development of *Hymenolepis nana* larvae in immunosuppressed mice. *Journal of Helminthology* **54,** 75–82.

Machnicka, B. & Smyth, J.D. (1985) The early development of larval *Taenia saginata in vitro* and *in vivo*. *Acta Parasitolica Polonica* **30,** 47–52.

Mitchell, G.F., Goding, J.W. & Rickard, M.D. (1977) Studies on immune responses to larval cestodes in mice. Increased susceptibility of certain strains and hypothymic mice to *Taenia taeniaeformis* and analysis of passive transfer of resistance with serum. *Australian Journal of Experimental Biology and Medical Science* **55,** 165–186.

Stevenson, P. (1983) Observations on the hatching and activation of fresh *Taenia saginata* eggs. *Annals of Tropical Medicine and Parasitology* **77,** 399–404.

Wang, I.C., Ma, Y.X., Guo, J.X. & Fan, P.C. (1997) A comparative study on egg hatching methods and oncosphere viability determination for *Taenia solium* eggs. *International Journal for Parasitology* **27,** 1311–1314.

Yang, X., Xu, H., Cao, Z. & Wang, W. (1994) Establishment of mouse model for *Cysticercus cellulosae*. *Chinese Journal of Parasitology and Parasitic Diseases* **12,** 297–299 (in Chinese with English summary).

(Accepted 28 October 1998)