Human *Taenia* eggs develop into cysticerci in *scid* mice

A. ITO[1]*, W. C. CHUNG[2], C. C. CHEN[3], M. ITO[4], S. ENDO[4], M. OKAMOTO[5] *and* P. C. FAN[3]

[1]*Department of Parasitology, Gifu University School of Medicine, Gifu 500, Japan*
[2]*Department of Parasitology, Taipei Medical College, Taipei 250, Taiwan, Republic of China*
[3]*Department of Parasitology, National YangMing University, Taipei 112, Taiwan, Republic of China*
[4]*Central Institute for Experimental Animals, Kawasaki 216, Japan*
[5]*Institute of Experimental Animal Sciences, Osaka University Medical School, Suita 565, Japan*

(*Received 3 June 1996; revised 16 July 1996; accepted 16 July 1996*)

SUMMARY

The intermediate hosts for *Taenia saginata* and *T. solium* are cattle and pigs (and humans for the latter), respectively. *In vitro*-hatched (but not activated) oncospheres of both Asian *Taenia* (*T. saginata asiatica*, a new subspecies of *T. saginata* or *T. asiatica*, a new species) and *T. solium* injected subcutaneously into the backs of mice with severe combined immunodeficiency (*scid*) developed into fully matured cysticerci. Five-month-old metacestodes of Asian *Taenia* had no hooklets and were bigger in size than those previously reported and similar to those of *T. saginata*. Their morphology suggested that the cysticerci were more advanced than those in the intermediate host animals. It is suggested that *scid* mice are valuable experimental animal models for studying human taeniid cestode infections.

Key words: Asian *Taenia*, *Taenia solium*, *Taenia saginata*, metacestodes, oncospheres, *scid* mice.

INTRODUCTION

Taenia saginata and *T. solium* are well-known human taeniid cestodes. Recently, Fan (1988) reported a unique Asian *Taenia* which, like *T. solium*, requires pigs as the intermediate host. The metacestode recovered from pigs has hooklets like *T. solium* but adult worms from humans have no hooklets like *T. saginata*. Regarding the taxonomy of this species, two ideas suggest that it should be a new subspecies of *T. saginata asiatica* (Fan, 1988; Fan *et al.* 1995; Zarlenga *et al.* 1991; Bowles & McManus, 1994) and a new species, *T. asiatica* (Eom & Rim, 1992, 1993). Despite the taxonomic issue, these cestodes require 2 mammalian hosts for completion of their life-cycle. The intermediate hosts for *T. saginata* and for both *T. solium* and Asian *Taenia* are mainly cattle and pigs, respectively. However, various mammals including monkeys, rabbits, bears, foxes, rats etc were reported to be infected with cysticerci having hooklets (reviewed by Gemmell *et al.* 1983). Recent experimental work by Fan and his colleagues has revealed that pigs as well as cattle and reindeer, and cattle, goats, monkey and wild boar as well as pig may be the intermediate host for *T. saginata* and Asian *Taenia*, respectively. Dogs and monkeys as well as pigs may act as the intermediate host for *T. solium* (Fan, Lin & Chen, 1992*a*; Fan, Lin & Chung, 1992*b*; Fan *et al.*, 1992*c*). The definitive host for these is exclusively human. Thus, the life-cycle of these taeniid cestodes is based on the food chains between cattle or pigs and humans who eat beef and pork (and viscera, mainly the liver of pigs) contaminated with cysticerci of these cestodes. As a consequence adult tapeworms develop in the intestine and contaminate the environment with eggs (embryonated eggs = oncosphere embryos in the embryophores) released from the adult worms. In *T. solium* infection, humans may suffer from cysticercosis cellulosae due to the accidental uptake of eggs (Gemmell *et al.* 1983; Schantz *et al.* 1992). The specificity of the definitive host appears to be very critical (Verster, 1974; Allan *et al.* 1991). Therefore, we have limited critical information on these human *Taenia* which are the most important causative

Table 1. Routes, sources and fates of eggs or oncospheres of taeniid species in *scid* mice

(Egg = oncosphere in embryophore. Oncospheres were inoculated orally and subcutaneously into the same individuals.)

Inoculation	Development of cysticerci in *scid*		
Route	With	Asian *Taenia*	*T. solium*
Oral	Eggs	No (0/4)	No (0/4)
Oral	Oncospheres	No (0/5)	No (0/5)
Subcutaneous	Oncospheres	Yes (5/5)	Yes (5/5)

* Corresponding author: Department of Parasitology, Gifu University School of Medicine, Gifu 500, Japan. Tel: +81 58 265 1241 Ext 2252. Fax: +81 58 267 2960. E-mail: akiraito@cc.gifu-u.ac.jp.

agents for both public health problems and economic loss. If we can establish some experimental animal models for cattle or pigs, it would be a considerable advance for (i) developing new research work on applied science in immunology such as vaccine development and immunodiagnosis in live-stock, humans and even wild animals to prevent cysticercosis, (ii) obtaining information on environmental contamination, or (iii) basic science in developmental biology, immunology and co-evolution in the host–parasite relationship etc.

In the present paper, we describe how to establish the fully developed cysticerci in mice with severe combined immunodeficiency (*scid* mice) instead of pigs for 2 species of human *Taenia*, *T. solium* and Asian *Taenia*.

MATERIALS AND METHODS

Animals

A total of 18, 8-week-old female mice with severe combined immunodeficiency (*scid*; C.B-17-*scid*), produced at the Central Institute for Experimental Animals, Kawasaki, Japan, were used for experimental infections (Ishiwata *et al.* 1992). The *scid* mice were infected with eggs or oncospheres orally or subcutaneously in a safety cabinet. They were kept in dry-heated (200 °C for more than 2 h) metal cages with autoclaved woody bedding and covered with a filter cap (CM-1-A, CLEA-Japan). Autoclaved drinking water and cobalt 60-irradiated food (CL-2, CLEA-Japan) were provided *ad libitum*. All materials and waste were autoclaved after use. All mice were autopsied under ether anaesthesia 5 months after oral or subcutaneous inoculation according to the institutional guidelines.

Parasites

Eggs of Asian *Taenia* were prepared in Taiwan just 1–2 weeks before experiments, whereas those of *T. solium* were collected in China and prepared in Taiwan approximately 2 months before. Preparation of eggs and *in vitro* hatching of oncospheres were carried out according to previously described methods (Takemoto *et al.* 1995; Lightowlers *et al.* 1984; Negita & Ito, 1994). Eggs or oncospheres, rinsed with sterile PBS, adjusted to approximately $1 \times 10^4/0{\cdot}2$ ml sterile PBS, were used for oral or subcutaneous inoculation.

RESULTS

The results are summarized in Table 1 and in Fig. 1. Oncospheres of these two human *Taenia* species inoculated subcutaneously developed into fully matured cysticerci under the dorsal skin of *scid* mice (Fig. 1 A–C). In contrast, when *scid* mice were orally inoculated with either eggs or oncospheres, they harboured no cysticerci in the liver, peritoneal cavity or muscles. All cysticerci of Asian *Taenia* and *T. solium* had fully developed scoleces and remained viable 5 months after infection. The number of cysticerci ranged from 34 to 564 in Asian *Taenia*, whereas the range was 2 to 27 in *T. solium*. Microscopical observation revealed that the 5-month-old, cysticerci of Asian *Taenia* had no hooklets, although 1-month-old larvae in pigs had them. In contrast, those of 5-month-old *T. solium* had hooklets (pictures not shown).

DISCUSSION

This is the first report that metacestodes of human taeniid cestodes such as *Taenia solium* and Asian *Taenia* (= *T. saginata asiatica* or *T. asiatica*) form fully developed cysticerci in mice subcutaneously infected with *in vitro*-hatched (but not activated) oncospheres. There is only one paper by Machnicka & Smyth (1985) showing the very early post-oncospheral development of *T. saginata* in immunosuppressed mice. As shown in Fig. 1 F, cysticerci of Asian *Taenia* were larger than those reported in pigs (Fan *et al.* 1995) and the size appeared to be similar to those of *T. saginata*. Although cysticerci of Asian *Taenia* became calcified within a few months after oral egg infection in pigs (Fan *et al.* 1995), they were all viable and larger in *scid* mice after 5 months of subcutaneous infection with oncospheres than those recovered from pigs 1 to 2 months after oral egg inoculation. It is suggested that the size of the cysticercus in the intermediate mammalian host might be controlled by some immune response which can not kill the established larvae but restricts the parasite to a smaller size (Mitchell, Goding & Rickard, 1977; Lucas *et al.* 1980; Ito, 1985; Ishiwata *et al.* 1992; Dixon & Jenkins, 1995). Alternatively, we speculate that there might be some unknown animal which may be a more suitable intermediate host for Asian *Taenia* than the pig. If there are such suitable host animals other than domesticated animals, they might be some wild rodents.

In *Echinococcus granulosus* infection, mice may be a good experimental animal model for human or cattle: activated oncospheres inoculated either intraperitoneally or intravenously developed into cysts in the peritoneal cavity or lung, thoracic cavity or liver (Dempster *et al.* 1991). We have used immediately *in vitro*-hatched oncospheres without activation of the oncospheres. So, it would be interesting to know if *in vitro*-hatched but non-activated oncospheres of *E. granulosus* subcutaneously inoculated into mice could

https://doi.org/10.1017/S0031182096008074 Published online by Cambridge University Press

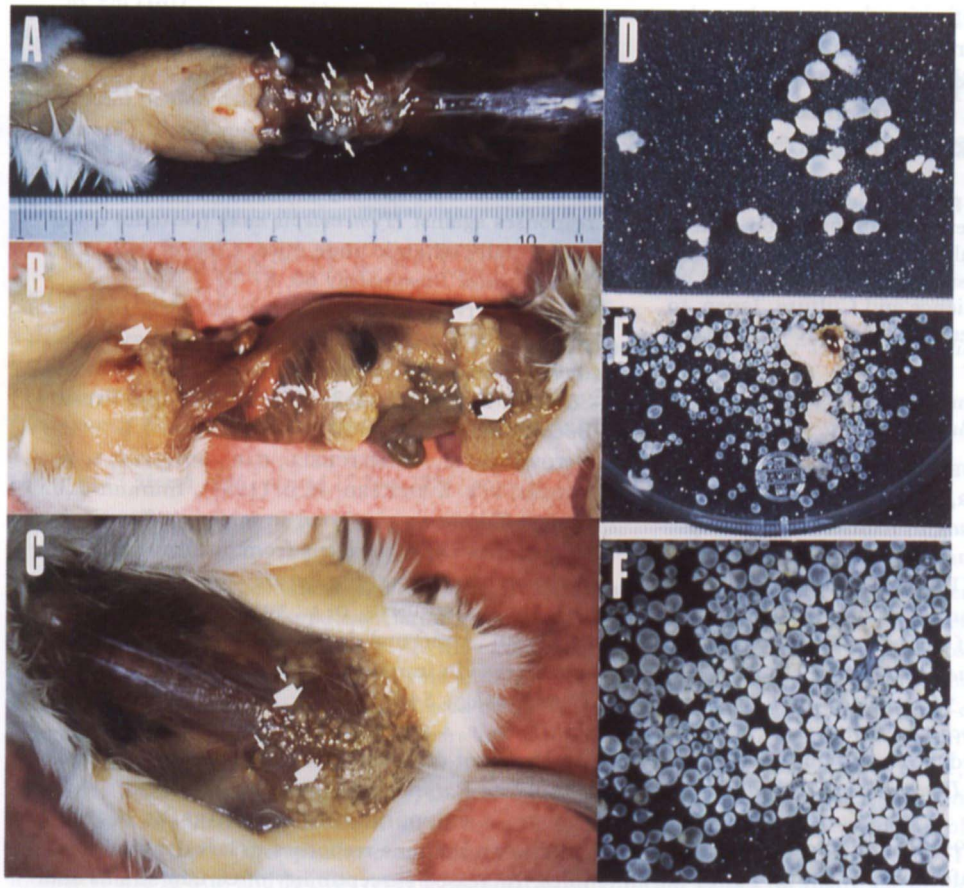

Fig. 1. Development of cysticerci of *Taenia solium* (A and D) and Asian *Taenia* (B,C, E and F) in *scid* mice. Small arrows show individual cysticerci, whereas large arrows show massive ones under the dorsal skin of *scid* mice. The smallest cysticerci of Asian *Taenia* (E and F) appeared to be very similar to those recovered from pigs (Fan *et al.* 1992*a*, 1995). D and E were under the same magnification; F was a higher magnification of E. D and E show cysticerci of *T. solium* and Asian *Taenia*, recovered from the mouse in A and B, respectively.

develop into cysts. *In vitro*-hatched but non-activated oncospheres of these human *Taenia* can develop into cysticerci in some tissues beyond the usual tissue specificity, provided that they are inoculated into such tissues. This was found to be true for *T. taeniaeformis* in *scid* mice, when *in vitro*-hatched oncospheres without activation were intraperitoneally infected (Ito *et al.* unpublished observations). Although we have no critical evidence on the importance of activation or the importance of the bile salt for activation, it is evident that oncospheres could be activated (like those of *Hymenolepis* spp.?) and develop into metacestodes under some experimental conditions. The maximum number of cysticerci of Asian *Taenia* recovered from each mouse was 564. We believe that we could obtain more cysticerci from each mouse if we could inject more oncospheres in a larger volume of PBS to maintain the space for metacestode growth. If we could use activated oncospheres, we might obtain higher numbers of metacestodes in *scid* mice.

A more interesting finding was that the 5-month-old, cysticerci of Asian *Taenia* had no hooklets, whereas 1-month-old larvae in pigs had them (Fan, 1988; Fan *et al.* 1992*a*,*b*, 1995). So, it is conceivable that the hooklets of Asian *Taenia* disappear or are lost from the scolex between 1 and 5 months of larval age.

Although we had no opportunity to obtain classical *T. saginata*, which requires cattle as the intermediate host, we do not doubt that the oncospheres would develop into cysticerci in *scid* mice, since activated oncospheres of this parasite could attain very early larval development in immunosuppressed mice (Machnicka & Smyth, 1985). It would be very interesting to determine whether 1-month-old cysticerci of either Asian *Taenia* or *T. saginata* in pigs or cattle and/or in *scid* mice do or do not have hooklets in order to obtain more critical information on the gene control for the hooklets through development and evolution of these human taeniid cestodes.

In this study, we have established *scid* mice as an experimental animal model for the intermediate host of human taeniid cestodes. This model can be used as a good *in vivo* incubator in checking the viability of taeniid eggs, preparation of a standardized larval stage to produce antigens for serodiagnosis, tools for comparative study on developmental biology with or without the immune system, a source of exper-

https://doi.org/10.1017/S0031182096008074 Published online by Cambridge University Press

imental infection in the definitive hosts, and in the establishment of an experimental animal model for human (reviewed by Ito & Smyth, 1987). Further experiments using several mutant mice including transgenic mice are in progress.

This work was supported in part by the Nissan Science Foundation, the Uehara Memorial Foundation and a grant-in-aid for international collaboration project on echinococcosis and cysticercosis (06044089, 07044243) by the Ministry of Education, Science and Culture, Japan, to A. Ito and by the National Science Council, ROC (NSC85-2331-B010-032) to P. C. Fan.

REFERENCES

ALLAN, J. C., GARCIA-DOMINGUEZ, C., CRAIG, P. S., ROGAN, M. T., LOWE, B. S. & FLISSSER, A. (1991). Sexual development of *Taenia solium* in hamsters. *Annals of Tropical Medicine and Parasitology* **85**, 573–576.

BOWLES, J. & McMANUS, D. P. (1994). Genetic characterization of the Asian *Taenia*, a newly described taeniid cestode of humans. *American Journal of Tropical Medicine and Hygiene* **50**, 33–44.

DEMPSTER, R. P., BERRIDGE, M. V., HARRISON, G. B. L. & HEATH, D. D. (1991). *Echinococcus granulosus*: development of an intermediate host mouse model for use in vaccination studies. *International Journal for Parasitology* **21**, 549–554.

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

EOM, K. S. & RIM, H. J. (1992). Experimental human infection with Asian *Taenia saginata* metacestodes obtained from naturally infected Korean domestic pigs. *Korean Journal of Parasitology* **30**, 21–24.

EOM, K. S. & RIM, H. J. (1993). Morphologic descriptions of *Taenia asiatica* sp. n. *Korean Journal of Parasitology* **31**, 1–6.

FAN, P. C. (1988). Taiwan *Taenia* and taeniasis. *Parasitology Today* **4**, 86–88.

FAN, P. C., LIN, C. Y. & CHEN, L. M. (1992*a*). Experimental infection of *Taenia saginata* (Burma strain) in domestic animals with special reference on its morphological characteristics. *Annals of Tropical Medicine and Parasitology* **86**, 317–318.

FAN, P. C., LIN, C. Y. & CHUNG, W. C. (1992*b*). Experimental infection of Philippine *Taenia* in domestic animals. *International Journal for Parasitology* **22**, 235–238.

FAN, P. C., CHUNG, W. C., LIN, C. Y. & PAWLOWSKI, Z. S. (1992*c*). Experimental infection with *Taenia saginata* (Poland strain) in Taiwanese pigs. *Journal of Helminthology* **66**, 198–204.

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.

GEMMELL, M. A., MATYAS, Z., PAWLOWSKI, Z. & SOULSBY, E. J. L. (1983). *Guidelines for Surveillance, Prevention and Control of Taeniasis/Cysticercosis*, VPH/83.49, pp. 207, World Health Organization, Geneva.

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. & SMYTH, J. D. (1987). Adult cestodes. In *Immune Responses in Parasitic Infections: Immunology, Immunopathology, and Immunoprophylaxis* (ed. Soulsby, E. J. L.), vol. 2, pp.115–163. CRC Press, Boca Raton.

LIGHTOWLERS, M. W., MITCHELL, G. F., BOWTELL, D. D. L., ANDERS, R. F. & RICKARD, M. D. (1984). Immunisation against *Taenia taeniaeformis* in mice: studies on the characterisation of antigens from oncospheres. *International Journal for Parasitology* **14**, 321–333.

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 Parasitologica 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.

NEGITA, T. & ITO, A. (1994). *In vitro* hatching of oncospheres of *Taenia taeniaeformis* using eggs isolated from fresh, frozen, formalin-fixed and ethanol-fixed segments. *Journal of Helminthology* **68**, 271–272.

SCHANTZ, P. M., MOORE, A. C., MUNOZ, J. L., HARTMAN, B. J., SCHEFER, J. A., ARON, A. M., PERSAUD, D., SARTI, E., WILSON, M. & FLISSER, A. (1992). Neurocysticercosis in an outhodox Jewish community in New York City. *New England Journal of Medicine* **327**, 692–695.

TAKEMOTO, Y., NEGITA, T., OHNISHI, K., SUZUKI, M. & ITO, A. (1995). A simple method for collecting eggs of taeniid cestodes from fresh, frozen or ethanol-fixed segments. *International Journal for Parsitology* **25**, 537–538.

VERSTER, A. (1974). The golden hamster as a definitive host of *Taenia solium* and *Taenia saginata*. *Onderstepoort Journal of Veterinary Research* **41**, 23–28.

ZARLENGA, D. S., McMANUS, D. P., FAN, P. C. & CROSS, J. H. (1991). Characterization and detection of a newly described Asian taeniid using cloned ribosomal DNA fragments and sequence amplification by the polymerase chain reaction. *Experimental Parasitology* **72**, 174–183.