# NOD-*scid* MOUSE AS AN EXPERIMENTAL ANIMAL MODEL FOR CYSTICERCOSIS

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**Abstract.** The major three species of human taeniid cestodes, *Taenia solium*, *T. saginata* and *T. saginata asiatica* (= *T. asiatica*) which require humans as the definitive host are still not rare in developing countries. Among these, *T. solium* is the most serious with medical and economic importance. Neurocysticercosis (NCC) in humans is now recognized as the major cause of neurologic disease in the world. As these human taeniid cestodes obligatory require domestic animals such as swine, cattle and swine as the major intermediate host animals respectively, it is not easy to analyze the basic research in these domestic animals. In this brief review, we introduce experimental animal model for these three species in order to obtain fully developed metacestode stage in severe combined immunodeficiency (*scid*) mice. Non-obese diabetic *scid* (NOD-*scid*) mice are expected to be a satisfactory animal model and to have advantages for analysis by several view points of developmental biology with gene expression throughout development, antigenic homology of cyst fluid of these three species, evaluation of drug efficacy or metacestocidal drug designs, confirmation of unknown taeniid gravid segments for identification based on the morphology and DNA analysis of metacestodes. The animal model is not only available for human *Taenia* spp but can also be applied to other taeniid cestodes of economic importance or in veterinary parasitology.

## INTRODUCTION

*Taenia solium*, *T. saginata* and *T. saginata asiatica* (= *T. asiatica*) are the three major human taeniid tapeworms require domestic animals such as pigs, cattle and pigs respectively, as the intermediate host, but it is not easy to use them for experimental research work. Table 1 is a brief summary of the intermediate host species for these three human *Taenia* spp and some others of economic importance. *T. solium* is the only species which causes neurocysticercosis (NCC) in humans (White, 1997; Schantz *et al*, 1998). WHO estimates that approximately 50,000 people die every year as indicated by Dr Urbani at this symposium (Urbani, 2000). Although it is still unclear if eggs of *T. saginata asiatica* can develop into metacestode (MC) stage in humans, it has been discussed by several researchers (Ito, 1992; Galan-Puchades and Fuentes 1999).

When we showed that *scid* mice were highly susceptible for oncospheres of *T. solium* and *T. saginata asiatica* (Ito *et al*, 1997b) and were able to develop into mature cysticerci, it was expected that they should be easily established in this animal model. There was a wider range or homology of the intermediate host species between *T. solium* and *T. saginata asiatica* than that of *T. saginata* (Table 1). Although *T. saginata* is less important medically, it is still important economically. In this review, we introduce (1) the brief history of such work, (2) how to carry out experimental infection, (3) the critical difference in susceptibility of the host (*scid*, nude and normal) mice, (4) the usefulness of *T. saginata* in *scid* mice and (5) what further studies can be done using this laboratory animal model.

## BRIEF HISTORICAL REVIEW

Classical research on the host specificity in the host-parasite relationship in cestode infections was reported in 1970s especially when congenitally athymic nude mice were introduced (Mitchell *et al*, 1980). They showed intra-strain variation of the host susceptibility and the usefulness of nude mice for analytical studies on the host resistance. Most work was on the host susceptibility to taeniid cestode infections under immunosuppressed or immunodeficient condition (reviewed by Lightowlers *et al*, 1993; Ito and Ito, 1999). Ito and others (1997a) reported that eggs of a rat-adapted *T. taeniaeformis*, which do not develop into MCs in immunocompetent mice, develop into fully developed MCs in *scid* female mice and the size of developed MCs appeared to be much bigger than those in nude mice. A breakthrough was that human *Taenia* develop into MCs in *scid* female mice, since *T. solium* and *T. saginata asiatica* require pigs as the obligative intermediate host (Ito *et al*, 1997b,c; Ito and Ito 1999). So far we have examined, female *scid* mice of C.B17-, BALB/c- or C57BL/6-origins are exclusively susceptible for oncospheres of these human taeniid species to develop into MCs, whereas males are all highly resistant with no MC development in these *scid* mice. However, such

Table 1
Major taeniid species with medical and economic importance and the mammalian intermediate host animals.

| Species | Mammalian intermediate host animals |
|---|---|
| *Taenia solium* | Pigs, dogs, baboon, humans |
| *T. saginata asiatica* (*T. asiatica*) | Pigs, cattle, goats, monkeys, wild boar, mice |
| *T. saginata* | Cattle, rain deer |
| *T. multiceps* | Sheep (humans) |
| *T. ovis* | Sheep |
| *T. hydatigena* | Sheep |
| *T. pisiformis* | Rabbits |
| *Echinococcus granulosus* | Sheep, goats, cattle, pigs, horse, buffaloes, camels, yaks, humans |
| *E. multilocularis* | Rodents, *Ochotona* spp, rabbits, yaks, pigs, humans |

sex dependent susceptible-resistant patterns in human *Taenia* infections (Ito and Ito, 1999) are not always rigid in such transgenic mice of different strain origin as C57BL/6-IA-ßKO (Gosgrove *et al*, 1991), C57BL/6-ß2-mKO (Zijlstra *et al*, 1989), C57BL/6-RAG2KO, BALB/c-RAG2KO (Shinkai *et al*, 1992). It is evident that *scid* female mice of C.B-17, BALB/c or C57BL/6 strains are highly susceptible to subcutaneous or intraperitoneal infection with *in vitro* hatched oncospheres of human taeniid species. So far we have examined, when eggs or *in vitro* hatched oncospheres are orally inoculated, they are not established as MCs. It might be a critical difference from eggs of *Echinococcus* spp or *T. taeniaeformis*, which can infect mice orally (reviewed by Ito and Ito, 1999).

**Preparation of *in vitro* hatched oncospheres for obtaining metacestodes (MCs) in *scid* mice**

There are several methods to obtain *in vitro* hatched oncospheres (Rajasekariah *et al*, 1980; Lightowlers *et al*, 1984; Smyth and McManus, 1989). The key for getting hatched oncospheres safely is to use 0.5% NaClO (sodium hypochloride) in PBS (not in distilled water)(Lightowlers *et al*, 1984 *vs* Ito *et al*, 1996).

1) Prepare viable eggs of taeniid cestodes.
2) Incubate and suspend eggs in 0.5 % NaClO in PBS (egg suspension in PBS is added with the same volume of 1.0 % NaClO in PBS) for approximately 5 minutes with air bubbling several times by pipetting.
3) Add the same volume of PBS into the *in vitro* hatched oncosphere suspension and centrifuge under 3,000 rpm, when almost all oncospheres have hatched after checking of it under microscope.
4) Repeat rinsing with sterile PBS several times.
5) Inject $1x10^3$ oncospheres in 0.5 ml into *scid* mice each subcutaneously, intraperitoneally, intravenously or intracranially depending on what and where we want to establish MCs in the animal.
6) Observe the animals at appropriate intervals.
7) Obtain fully developed metacestodes within 4 months after injection.
8) Infect experimental animals such as dogs or cats or monkeys for experimental maintenance of adult worms.

**Development of adult cestodes in unnatural mammalian host**

There are no reports on the success in the establishment of mature adult worms of human *Taenia* species in experimental animals except Chinchilla (Maravilla *et al*, 1998). In order to obtain adult cestodes in unnatural mammalian host animals, corticosteroid are used to initiate development (Moss, 1972; Verster, 1974; Ito, 1982; Ito and Kamiyama, 1984, 1986; Ito and Smyth, 1987; Sato and Kamiya, 1989; Kamiya and Sato, 1990; Maravilla *et al*, 1998). It has rather been easy to ascribe the effect of corticosteriod to immunosuppression. However, based on comparison of congenitally athymic nude animals etc (Ito and Kamiyama 1984, 1986) we commented that the effect was not always ascribed to immunosuppression but might be due to growth hormone (Moss, 1972; Ito and Smyth, 1987). We recognize that larval cestodes have receptors of steroid and coticosteroid which might directly affect the MCs differentiate and grow into adult stage in the unnatural host (Breham and Frosch, 2001).

**The usefulness of *T. saginata* and *T. saginata asiatica* (*T. asiatica*)**

Table 2 is a summary of our recent experimental work using several different strains of *scid* mice for infection of *T. solium*, *T. saginata asiatica* (*T. asiatica*) and *T. saginata* (Ito *et al*, 1997b, c, 2001). We have examined, female *scid* mice of C.B17-, BALB/c- and C57BL/6-origins and these appear to be highly

Table 2
Critical difference in susceptibility for human *Taenia* spp in males and females of several strains of *scid* mice.

| Mouse strain | Females | Males |
|---|---|---|
| *Taenia saginata asiatica* | | |
| C.B-17-*scid* | 16/16[a] | 0/6 |
| C57BL/6-*scid* | not tested | 0/3 |
| BALB/c-*scid* | 6/6 | 0/3 |
| *T. solium* | | |
| C.B-17-*scid* | 5/5 | NT |
| *T. saginata asiatica* | | |
| NOD-*scid* | 21/21 | 17/17 |
| C.B-17-*scid* | 10/10 | 0/10 |
| *T. saginata* | | |
| NOD-*scid* | 15/15 | 18/18 |
| C.B-17-*scid* | 10/10 | 0/10 |

[a]: Number of mice harboring metacestodes/Number of mice infected with approximately 1 x $10^3$ oncospheres/0.5 ml sterile PBS.

susceptible to percutaneous injection of *in vitro* hatched oncospheres of these human taeniid cestodes. However, most recently when we used NOD (nonobese diabetic)-*scid* mice, both sexes became highly susceptible and all individuals harbored many well developed MCs under the back skin. There is, however, a significant difference in the number of established MCs between female and male NOD-*scid* (Ito *et al*, in prep). The MCs of both *T. saginata asiatica* and *T. saginata* developed to approximately 10 mm in diameter and the cyst membrane was very fragile in the former compared with that in the latter. It should be pointed out that the size of MC of *T. saginata asiatica* becomes large and similar to that of *T. saginata* and that MCs of both species survive as infective stage in NOD-*scid* mice as long as the mice survive (from 4 – 12 months so far examined). Although it usually survive only a few months and remains 2-3 mm in diameter in the liver of pigs (Fan, 1988). In order to obtain conclusive information on the unique high susceptibility in NOD-*scid* mice, we are now carrying out direct comparison of the number and size of MCs developed in males and females of NOD-*scid*, of NOD and of C.B-17-*scid*. After obtaining the basic critical information on the sex difference in the susceptibility to *Taenia* infections in these mice, we are planning to analyze the mechanism.

Although the mechanisms of the host specificities or sex differences in susceptibility to the experimental infections with these taeniid cestodes are still unknown, we can use these unique mice, especially NOD-*scid* mice for some experimental studies. Analysis of several view points of developmental biology with gene expression throughout development, antigenic homology of cyst fluid of these three species, evaluation of drug efficacy or metacestocidal drug designs, confirmation of unknown taeniid gravid segments for identification based on the morphology and DNA analysis of metacestodes. The animal model is also expected not only available for human *Taenia* spp but also for other taeniid cestodes such as *T. ovis*, *T. hydatigena* or *T. multiceps* etc of economic importance and veterinary parasitology.

## PROSPECTS

We have found that these three taeniid species easily develop into fully developed MC stage in *scid* mice. We introduce the most recent advances on this line of experimental work using several strains of *scid*, congenitally athymic, nude and immunocompetent normal mice. *T. solium* and *T. saginata asiatica* are expected to have a wider spectrum of the intermediate host specificity than *T. saginata* and therefore are expected to develop in such immunodeficient mice. There is no experimental data on the development of metacestodes of *T. saginata* developed in *scid* mice. In this review paper, we have introduced (i) the uniqueness of NOD-*scid* mice, (ii) the critical sex difference in the susceptibility and resistance to these cestode infections between NOD-*scid* and C.B-17-*scid* or other strains of *scid* mice, (iii) what we can do using this animal model. We also summarize methods to carry out experimental infections using viable eggs of these taeniid species. We expect that NOD-*scid* mice to become (a) supreme experimental animal models for cysticercosis and (b) highly useful for analytical

work in developmental biology with stage specific gene expressions, in molecular phylogeny of these cestodes and evolution, in critical evaluation of antigenic components of cyst fluid of these taeniid species, and evaluation of metacestocidal drugs etc in the future (Ito *et al*, 2001).

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