[Jpn. J. Parasitol., Vol. 45, No. 1, 1–5, February, 1996]

Acquired Resistance against Adult *Echinococcus multilocularis* Infection Observed in Golden Hamsters

JUNKO INOHARA[1)], NARIAKI NONAKA[1)], HONG KEAN OOI[2)], YUZABURO OKU[1)] AND MASAO KAMIYA[1)]

[1)]*Laboratory of Parasitology, Department of Animal Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan.*
[2)]*Department of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan, R.O.C.*
(Accepted January 16, 1996)

Abstract

Acquired resistance against adult *Echinococcus multilocularis* infection was studied using golden hamsters harboring adult worms less than 25 days post-inoculation (PI). Sixteen, male 4-week-old golden hamsters were divided into 3 groups. Group A was orally inoculated with 20,000 viable protoscoleces followed by oral treatment with praziquantel at 23 and 25 days PI and then challenged orally with 20,000 viable protoscoleces at 40 days PI. Group B was intraperitoneally inoculated with 20,000 viable protoscoleces, then treated with praziquantel and challenged as described for group A. Group C served as control group without previous inoculation of protoscoleces, but was given praziquantel and then challenged with similar infection dose as described for group A. All hamsters were killed at 5 days after challenge inoculation. The mean numbers and standard deviation (SD) of adult worms recovered in groups A, B and C were 30.2±17.9, 103.7±45.1 and 594.6±71.2, respectively. Significant differences were recognized between groups A and C and between groups B and C using Scheffe's test ($P<0.001$). Specific serum IgG measured by an indirect ELISA using *E. multilocularis* somatic antigen was clearly elevated in group A and B. These findings indicate that hamsters with previously established infection acquired resistance and the level of specific IgG was also increased in their sera. This study suggests that the alternative definitive host model using golden hamsters could be useful for immunological studies related to assessment of vaccines.

Key words: *Echinococcus multilocularis*; golden hamster; acquired resistance; antibody response; experimental model.

Introduction

Although the importance of vaccination of definitive hosts has been widely discussed in the control of hydatidosis, immunological studies of *Echinococcus* infection in the definitive host were not adequately carried out (Smyth and McManus 1989). Some degree of protection to *E. granulosus* has been reported by several workers, however, these results are still controversial (Gemmell, 1962; Movsesijan and Mladenovic, 1970; Herd, 1977; Aminzhanov, 1980; Gemmell *et al.*, 1986). In the case of *E. multilocularis* in which animal hosts are mainly feral animals, such as foxes and wild rodents, work has not been reported on acquired immunity in definitive host. One of the reasons could be due to the difficulty of using dogs and/or foxes for experimental infection. Recently, alternative definitive host model has been established using immunosuppressed Mongolian gerbils (*Meriones unguiculatus*) and golden hamsters (*Mesocricetus auratus*) (Kamiya and Sato 1990a, b). It was also reported that without immunosuppression, adult *E. multilocularis* were harbored for a restricted period, (less than 25 days post-inoculation) in golden hamsters (Kamiya and Sato, 1990b). Accordingly, immunocompetent golden hamsters may be useful for

Correspondence: Masao Kamiya, Kamiya@vetmed.hokudai.ac.jp.
猪原潤子[1], 野中成晃[1], 黄　鴻堅[2], 奥　祐三郎[1], 神谷正男[1] ([1]北海道大学大学院獣医学研究科動物疾病制御学講座, [2]台湾国立中興大学獣医学系)

The present work was supported, in part, by Scientific Research Grant from the Ministry of Education, Science and Culture, Japan (nos. 06041005, 06454120 and 07557204),

immunobiological studies on adult *E. multilocularis* infection. Using this model, acquired resistance against adult *E. multilocularis* infection was studied.

Materials and Methods

Parasites

Protoscoleces of *E. multilocularis* were originally isolated from gray red-backed voles (*Clethrionomys rufocanus bedfordiae*) at Tobetsu, Hokkaido in 1992 and have since been maintained in our laboratory by intraperitoneal passage using Mongolian gerbils.

Animals and infection

Sixteen, male 4-week-old golden hamsters purchased from a commercial breeder (Japan SLC, Hamamatsu, Japan), were used. Individual animals were kept in individual plastic cage, fed commercial pelleted food (CE-2; Clea Japan, Tokyo, Japan) and given water *ad libitum*. The hamsters were divided into 3 groups. Group A consisting of 5 animals was orally inoculated with 20,000 viable protoscoleces followed by oral treatment with 5mg/Kg b.w. praziquantel (Droncit®; Bayer, Levkusen, Germany) at 23 and 25 days post-primary inoculation (PI) for complete expulsion of all worms, and then challenged orally with 20,000 viable protoscoleces at 40 days PI. The establishment of primary infection and effect of praziquantel deworming were confirmed by detection of coproantigen in the feces using the sandwich ELISA method of Sakashita *et al.* (1995). Briefly, rabbit polyclonal antibody against excretory/secretory antigen of *E. multilocularis* was used to capture coproantigen in the feces. This was followed by the addition of mouse monoclonal antibody (designated Em A9) against somatic antigen of adult *E. multilocularis*. Then horseradish peroxidase conjugated rabbit-anti-mouse IgG, A, M. (ZYMED, San Francisco, U.S.A.) as secondary antibody and ortho-phenylenediamine and H_2O_2 as substrate were added for color development. Absorbance at 490nm was measured using an ELISA reader (Model 450 microplate reader; Bio-Rad, California, U.S.A.). Coproantigen of *E. multilocularis* were detected in the hamster feces from 7 to 14 days PI. However, from 21 to 40 days PI, the coproantigen titer decreased to zero. Group B consisting of 6 animals was intraperitoneally inoculated with 20,000 viable protoscoleces, treated with praziquantel and challenged as described for group A. Group C consisting of 5 animals served as control group. These animals were not given the primary infection of protoscoleces, but was administered praziquantel and then challenged as described for group A.

Autopsy and recovery of worms

All hamsters were sacrificed using diethyl ether at 45 days PI (5 days after challenge), and the intestinal mucosa was scraped and fixed in 10% formalin solution. The number of worms was counted under a dissection microscope.

Preparation of somatic antigen for ELISA

E. multilocularis protoscoleces somatic antigen was made from the same batch of cysts used for primary inoculation. Protoscoleces from gerbil were washed three times in phosphate buffered saline (PBS, pH 7.4) and stored at –40°C until use. The stored protoscoleces were thawed in PBS containing the following protease inhibitors; 5mM ethylenediamine tetraacetic acid disodium salt (Nakarai, Kyoto, Japan), 5mM iodoacetamide (Wako, Osaka, Japan), 1mM phenylmethylsulfonyl fluoride (Wako), 0.1mg/ml pepstatin A (Sigma, St. Louis, U.S.A.) and 5mM 1,10-phenanthroline monohydrate (Wako) as well as 0.1% Triton X-100 (Wako). The worms were then homogenized with a teflon homogenizer, and sonicated for 5 minutes in an ice water bath at 0°C. After three times repeated freezing (–40°C) and thawing (4°C), the homogenate was centrifuged at 10,000×g for thirty minutes at 4°C. The supernatant was used as somatic antigen.

Detection of specific serum IgG

To measure the specific serum IgG titer, blood was collected from hamsters by orbital plexus puncture using capillary glass under ether anesthesia at 0, 7, 14, 21, 28 and 40 days PI and also by cardiac puncture at 45 days PI. Sera were separated and stored at –20°C until use. Specific serum IgG against somatic antigen of protoscoleces was detected by an indirect ELISA in which the somatic antigen was coated onto microplate and horseradish peroxidase-

conjugated goat-anti-hamster IgG (Cappel, Pennsylvania, U.S.A.) as secondary antibody was added followed by ortho-phenylenediamine and H_2O_2 as substrate for color development. The absorbance value at 490nm was measured with an ELISA reader (Model 450 microplate reader; Bio-Rad).

Statistical analysis

The Scheffe's test (Weerahandi, 1995) was used to analyse the significant differences among the number of worms recovered from the different groups of hamsters.

Results

The mean number and SD of adult *E. multilocularis* recovered at 45 days PI (5 days after challenge) in groups A, B and C were 30.2±17.9, 103.7±45.1 and 594.6±71.2, respectively (Fig. 1). Significant differences ($P<0.001$) were seen between groups A and C and between groups B and C by Scheffe's test. However, there was no significant difference in the mean number of worms between groups A and B. In group A, it was obvious that previously inoculated worms were expelled from the small intestine of hamsters because no differences in state of growth among recovered worms was observed. In group B, some of the intraperitoneally inoculated protoscoleces transformed into small cysts, but almost all were embedded in a purulent exudate. The Peyer's patches and mesenteric lymph node were larger in groups A and B, than group C.

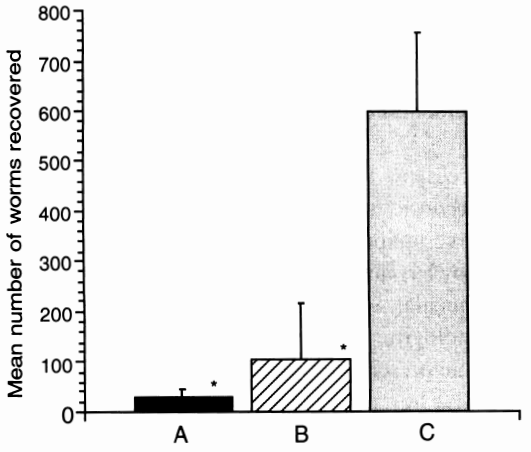

Fig. 1 Mean numbers of adult *Echinococcus multilocularis* recovered from golden hamsters at 5 days after oral challenge inoculation with 20,000 protoscoleces. Error bars represent standard deviations of each group. A; n=5, inoculated with 20,000 protoscoleces orally 40 days before challenge inoculation. B; n=6 inoculated with 20,000 protoscoleces intraperitoneally 40 days before challenge inoculation. C; n=5 controls. Asterisks (*) indicate significant differences from controls by Scheffe's test ($p<0.001$).

Specific serum IgG against somatic antigen of protoscoleces was detected from 14 days PI in group A, and higher response to challenge inoculation (at 45 days PI) was obtained compared to group B. In group B, however, specific serum IgG was detected from 7 days PI, which is earlier than that of group A, and the OD value reached plateau at 28 days PI. The serum antibody response to challenge infection was not detected (Fig. 2).

Discussion

Production of specific serum antibodies has been reported in dogs and foxes orally inoculated with *E. granulosus* and *E. multilocularis* (Jenkins and Rickard, 1986; Barriga and Al-Khalidi, 1986; Gasser *et al.*, 1993; Gottstein *et al.*, 1991). These observations indicated that oral inoculation of protoscoleces could sufficiently stimulate the host immune system in the natural definitive hosts. However, little is known about the relationship between antibody response and expulsion of worms. Immunological observations of mucosal membrane suggests that local humoral and cellular reactions are important for the protection against pathogenetic agents (Bienenstock and Befus, 1980). Deplazes *et al.* (1994) suggested the significance of local immune response by demonstrating parasite-specific antibody in Peyer's patches cell culture derived from *E. granulosus* infected dogs, in contrast, cells from mesenteric and popliteal lymph nodes or from uninfected dogs neither produced antibodies whilst in *in vitro* cultures.

Our results showed that acquired resistance could be established in hamsters either by oral or intraperitoneal inoculation of vigorous protoscoleces, at the same time, elevation of specific serum IgG was

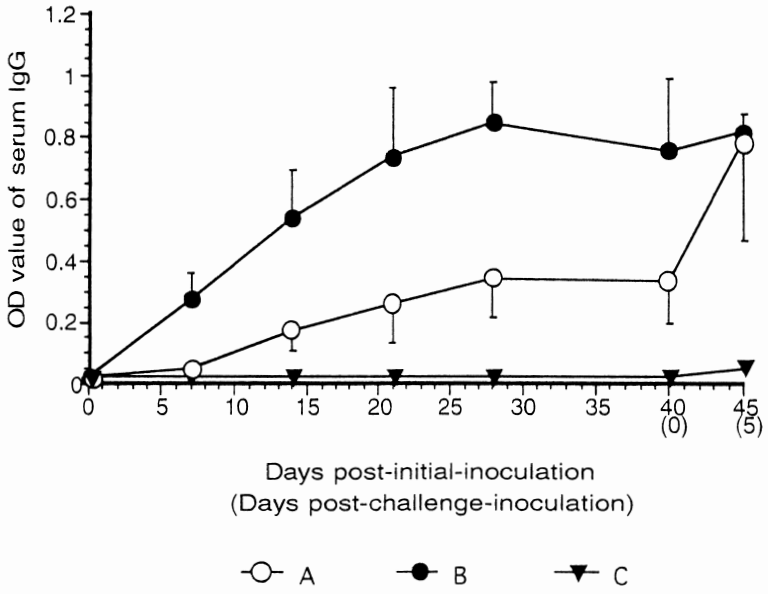

Fig. 2 Detection of serum IgG against *Echinococcus multilocularis* antigen in infected hamsters. The mean optical density (OD) value of ELISA and standard deviations in each group are shown. Group A; n=5, inoculated with 20,000 protoscoleces orally 40 days before challenge inoculation. B; n=6 inoculated with 20,000 protoscoleces intraperitoneally 40 days before challenge inoculation. C; n=5 controls, no previous inoculation except for challenge infection.

observed. These systemic humoral responses suggest that primary infection with *E. multilocularis* stimulated host immunity. Further work is needed to investigate the relationship between host antibody response and expulsion of worms from immunized hamsters. Moreover we observed that Peyer's patches and mesentric lymph nodes were larger in challenged groups with protoscoleces (group A and B) than in the group without previous inoculation (group C). Not only cells of the Peyer's patches but also the cells of the mesentric lymph nodes may play an important role in acquired immunity against protoscoleces of *E. multilocularis* in hamsters.

Although the immunocompetent hamster model may not completely represent natural infection, the acquisition of acquired resistance produced by inoculation of protoscoleces suggests that natural definitive hosts could acquire sufficient resistance by immunization with appropriate immunogens. Of particular importance is that intraperitoneal inoculation of protoscoleces induced acquired resistance in golden hamsters. This suggests the possibility of parenteral immunization using protoscoleces as antigen.

Successful vaccination of definitive hosts of *Echinococcus*, such as dogs and foxes, would be an attractive option in the control of hydatid disease (Lightowlers and Rickard, 1993). Although further experimental work is necessary to understand the immunological background of the acquired resistance, our present study suggests that the alternative definitive host model using golden hamsters can be useful for immunological studies such as assessment of vaccination trials.

References

1) Aminzhanov, M. (1980): Immunoprophylaxis of hydatidosis in animals. Trudy Uzbek. Nauchno-issled. Vet. Inst., 30, 15–18 (in Russian).
2) Barriga, O. O. and Al-Khalidi, N. W. (1986): Humoral immunity in the prepatent primary infection of dogs with *Echinococcus granulosus*. Vet. Immunol. Immunopathol., 11, 375–389.

3) Bienenstock, J. and Befus, A. D. (1980): Mucosal immunology. Immunology, 41, 249–268.
4) Deplazes, P., Thompson, R. C. A., Constantine, C. C. and Penhale, W. J. (1994): Primary infection of dogs with *Echinococcus granulosus*: systemic and local (Peyer's patches) immune responses. Vet. Immunol. Immunopathol., 40, 171–184.
5) Gasser, R. B., Jenkins, D. J., Paolillo, E., Parada, L., Cabrera, P. and Craig, P. S. (1993): Serum antibodies in canine echinococcosis. Int. J. Parasitol., 23, 579–586.
6) Gemmell, M. A. (1962): Natural and acquired immunity factors interfering with development during the rapid growth phase of *Echinococcus granulosus* in dogs. Immunology, 5, 496–503.
7) Gemmell, M. A., Lawson, J. R. and Roberts, M. G. (1986): Population dynamics in echinococcosis and cysticercosis: biological parameters of *Echinococcus granulosus* in dogs and sheep. Parasitology, 92, 599–620.
8) Gottstein, B., Deplazes, P., Eckert, J., Muller, B., Schott, E., Helle, O., Boujon, P., Wolff, K., Wandeler, A., Schwiete, U. and Moegle, H. (1991): Serological (Em2-ELISA) and parasitological examinations of fox populations for *Echinococcus multilocularis* infections. J. Vet. Med. B, 38, 161–168.
9) Herd, R. P. (1977): Resistance of dogs to *Echinococcus granulosus*. Int. J. Parasitol., 7, 135–138.
10) Jenkins, D. J. and Rickard, M. D. (1986): Specific antibody responses in dogs experimentally infected with *Echinococcus granulosus*. Am. J. Trop. Med. Hyg., 35, 345–349.
11) Kamiya, M. and Sato, H. (1990a): Complete life cycle of the canid tapeworm, *Echinococcus multilocularis*, in laboratory rodents. FASEB J., 4, 3334–3339.
12) Kamiya, M. and Sato, H. (1990b): Survival, strobilation and sexual maturation of *Echinococcus multilocularis* in the small intestine of golden hamsters. Parasitology, 100, 125–130.
13) Lightowlers, M. W. and Rickard, M. D. (1993): Vaccination against cestode parasites. Immunol. Cell. Biol., 71, 443–451.
14) Movsesijan, M. and Mladenovic, Z. (1970): Active immunization of dogs against *Echinococcus granulosus*. Vet. Glas., 24, 189–193 (in Croatian).
15) Sakashita, M., Sakai, H., Kohno, H., Ooi, H-K., Oku, Y., Yagi, K., Ito, M. and Kamiya, M. (1995): Detection of *Echinococcus multilocularis* coproantigen in experimentally infected dogs using murine monoclonal antibody against adult worms. Jpn. J. Parasitol., 44, 413–420.
16) Smyth, J. D. and McManus, D. P. (1989): The physiology and biochemistry of cestodes. 1st ed., Cambridge University Press, Cambridge, 283–304.
17) Weerahandi, S. (1995): ANOVA under unequal error variance. Biometrics, 51, 589–599.