[Jpn. J. Parasitol., Vol. 44, No. 5, 404–412, October, 1995]

Development and Characterization of Murine Monoclonal Antibodies to *Echinococcus multilocularis* Adult Worms and Its Use for the Coproantigen Detection

HIROMI KOHNO[1)], HIROFUMI SAKAI[1)], MUNEHIRO OKAMOTO[2)], MAMORU ITO[3)], YUZABURO OKU[1)] AND MASAO KAMIYA[1)]

[1)]*Department of Animal Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan.*
[2)]*The Institute of Experimental Animal Sciences, Osaka University Medical School, Suita-shi 565, Japan.*
[3)]*Central Laboratory for Experimental Animals, Kawasaki, Japan.*
(Accepted December 1, 1995)

Abstract

Murine monoclonal antibodies (MoAbs) against *Echinococcus multilocularis* adult worms were produced and characterized. Elven MoAbs were obtained, of which, six were IgM (designated as EmA1, EmA2, EmA3, EmA4, EmA5 and EmA6) and five were IgG (EmA7, EmA8, EmA9, EmA10 and EmA11). The MoAbs were tested by indirect immunoperoxidase tissue staining (IP) using paraffin embedded sections of *E. multilocularis* adult worms. The MoAbs were classified into 7 types based on tissue staining pattern. All the MoAbs were also tested by ELISA against the antigens of *E. multilocularis* adult worms, metacestodes and other helminth parasites, such as *Taenia taeniaeformis, T. crassiceps, T. hydatigena, Spirometra erinaceieuropaei, Hymenolepis diminuta* and *Toxocara canis* adult worms. EmA9 reacted only to *E. multilocularis* adult worm antigens. Since EmA9 recognized antigens of the tegument and excretory canal of the worm, as well as host intestinal epithelium around the worms by IP, it is suggested that EmA9 recognized the excretory/secretory (E/S) antigens of the worm. Sandwich ELISA using rabbit polyclonal antibodies against somatic antigens of *E. multilocularis* adult worms and EmA9 was performed to detect the E/S products in feces, i.e. coproantigens, of experimentally infected dogs. In three infected dogs, the coproantigens were detected as early as 3 days postinfection (PI) and continued to be positive until autopsy day 20 PI.

Key words: *Echinococcus multilocularis*; monoclonal antibody; coproantigen; ELISA; immunodiagnosis.

Introduction

Larval infection with *Echinococcus multilocularis* causes one of the most important zoonoses, since infection in humans is often fatal. Red foxes, *Vulpes vulpes schrencki*, are considered as the most common definitive host in Hokkaido, Japan, however, domestic dogs are very important in the transmission because of their close relationship to humans. Therefore development of reliable diagnostic methods for detecting infected dogs is necessary when contemplating on a control program.

Recently, some immunological methods has been proposed for the diagnosis of *Echinococcus* infected dogs. Specific antibodies has been detected in sera of dogs infected with *Taenia* spp. or *Echinococcus* spp. (Heath *et al.*, 1985; Jenkins and Rickard, 1985, 1986; Gasser *et al.*, 1988, 1993). It was shown that serodiagnosis using enzyme-linked immunosorbent assay (ELISA) is more effective than fecal examination. Coproantigen detection of *Echinococcus, Taenia* and *Hymenolepis* in the host feces has been also studied (Allan and Craig, 1989; Allan *et al.*, 1990, 1992; Deplazes *et al.*, 1990, 1991, 1992; *Maas* et al., 1991, 1992). These studies showed that sandwich ELISA using polyclonal antibodies against the para-

Correspondence: Masao Kamiya
河野尋美[1], 酒井博史[1], 岡本宗裕[2], 伊藤　守[3], 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]大阪大学医学部附属動物実験施設, [3](財)実験動物中央研究所)

The present work was supported, in part, by Scientific Research Grant from the Ministry of Education, Science, Sport and Culture, Japan (nos. 63480086, 63044005 and 02404083).

site antigens is able to detect coproantigens, and that it is specific to the genus level and reflect current infection.

Murine monoclonal antibodies (MoAbs) against *E. multilocularis* metacestodes antigens were produced and production protocols published in several reports (Craig *et al.*, 1981; Chamech *et al.*, 1990; Gottstein *et al.*, 1992). MoAbs against *E. multilocularis* oncospheral antigens were also produced and used for the differentiation of taeniid eggs in feces of dogs (Craig *et al.*, 1986). Furthermore, MoAbs against the excretory/secretory (E/S) antigens of *T. saginata* metacestodes has been used for the detection of circulating antigens in infected cattle (Harrison *et al.*, 1989; Brandt *et al.*, 1992). However, the production of MoAbs against *E. multilocularis* adult worm antigens and/or their use for the immunodiagnosis of infected dogs, such as detection of coproantigen or circulating antigen, has not been described.

The purpose of the present work is to develop MoAbs against *E. multilocularis* adult worm antigens for immunodiagnostic use in infected definitive hosts and characterize the antigens recognized by these MoAbs. This paper describes the distribution of the antigens recognized by MoAbs, the immunoblotting analysis and cross-reaction test using various parasites antigens in order to select MoAbs with immunodiagnostic potential. Furthermore, we also describe the coproantigen detection in feces of experimentally infected dogs using a selected MoAb.

Materials and Methods

Antigens

Somatic antigens for immunization to mice and rabbit were prepared as follows: *E. multilocularis* adult worms recovered from experimentally infected dogs were washed several times with Tris-HCl buffer (0.05 M, pH 7.8) and homogenized using glass/teflon homogeniser with addition of Tris-HCl buffer containing 1% sodium deoxycholate (Tris-DOC). Homogenized material was stirred in Tris-DOC buffer for 24 hours at 4°C and then centrifuged 10,000 g for 30 min at 4°C. The supernatant was sufficiently dialysed in Tris-HCl buffer to remove DOC and used as antigen to immunize mice. Protein concentration of this antigen was assessed by a Bio-Rad protein assay kit using bovine γ-globulin as standard.

Somatic antigens of adult and larval stages of *E. multilocularis*, *Taenia taeniaeformis*, *T. crassiceps*, *T. hydatigena*, *Spirometra erinaceieuropai*, *Hymenolepis diminuta* and *Toxocara canis* adult worms were used for the evaluation of the specificity of MoAbs. Each antigen as prepared in the manner described above.

MoAb production

E. multilocularis antigen solution containing 1.0 mg protein/ml in saline was prepared. BALB/cAn mice were injected intraperitoneally with 100 μl of the antigen solution (containing 100 μg protein) and Freund's complete adjuvant (Nakarai Tesque, Kyoto, Japan) in 0.2 ml total volume (antigen solution: adjuvant = 1:1). Two weeks after the first injection, mice were given intraperitoneal booster injection with 100 μl of the antigen solution and Freund's incomplete adjuvant (Nakarai Tesque). Three days before fusion, mice were injected intravenously with 100 μl of the antigen solution. Mice were killed 3 days after last injection and the spleen removed under sterile conditions. Splenocytes were fused with NS-1 myeloma cells (provided by Dr. M. Ito, Central Laboratory for Experimental Animals) using a 50% polyethylene glycol 1500 (Boehringer Mannheim, W. Germany) solution in serum free DMEM (Gibco Laboratories, New York, U.S.A) containing benzylpenicillin potassium (10^5 U/l, Meiji, Tokyo, Japan) and streptomycin sulfate (0.1 g/l, Meiji). Fusion and cell-culture procedures were carried out as described by De St. Groth and Scheidegger (1980).

Supernatant fluids showing cell growth in microtiter wells were removed and assayed for antibody activity using ELISA with *E. multilocularis* somatic antigen. Positive wells were identified and the cells were collected and transferred into 24-well plate for further culture. After further cell growth, the supernatant fluids in each well were again assayed for antibodies. Cells in the positive wells were cloned by limiting dilution in DMEM with 10% FCS using a 96-well microtiter plate. BALB/cAn mice thymocytes were used as feeder cells. After a few days, the wells were again screened by ELISA.

Cells showing suitable growth and secreting antibodies against *E. multilocularis* somatic antigen were repeatedly recloned. Clones secreting antibodies against *E. multilocularis* somatic antigen were transferred back to 24-well plates for growth. After a few days, clones were transferred to petri dish and cultured.

Monoclonal antibodies were recovered from the cell culture supernatant.

*Rabbit anti-*E. multilocularis *somatic antigen antibody*

Rabbit antibody against *E. multilocularis* somatic antigens was prepared as described by Baumann and Gottstein (1987). Briefly, antigen solution containing 1.2 mg protein/ml in saline was prepared. Rabbits were injected subcutaneously with 500 μl of the antigen solution (containing 600 μg protein) and Freund's complete adjuvant in 1 ml total volume (antigen solution: adjuvant = 1:1). Two weeks after the first injection, rabbits were given subcutaneous booster injection with 500 μl of the antigen solution and Freund's incomplete adjuvant. Two weeks after the second injection, rabbits were injected intravenously with 500 μl of the antigen solution. Ten days after, rabbits were bled and sera were collected. Immunoglobulin was precipitated using a half-saturated solution of ammonium sulfate and dialysed extensively against PBS. Immunoglobulin concentration was assessed as described above.

Immunoperoxidase staining of histological sections

Paraffin-embedded sections of *E. multilocularis* adult worms were treated basically as described by Nakane and Perse (1967). The section was mounted on 1% ovalbumin coated slide-glass and dried. After deparaffinization, the section was treated with methanol containing 1% H_2O_2 for 20 min in order to inhibit endogenous peroxidase activity of granulocytes and washed with phosphate buffered saline (PBS). For blocking, 2% normal rabbit serum in PBS was applied to the section and incubated for 20 min at room temperature. After washing with PBS, hybridoma culture supernatant was applied and incubated for 1 hour at room temperature. After further washing, horseradish peroxidase (HRP) conjugated goat anti-mouse IgG or HRP conjugated goat anti-mouse IgM (DAKO Japan Co., Ltd., Kyoto, Japan), diluted 1/200 with 2% normal rabbit serum in PBS, was applied and incubated for 1 hour at room temperature. The sections were washed overnight in PBS at 4°C and then treated with substrate solution; 0.02%, 3,3-diaminobenzidine (DAB, Dojindo Laboratories, Kumamoto, Japan), 0.02% H_2O_2 in Tris-HCl buffer (0.05 M, pH 7.6) for 10 min. To stop the reaction, sections were washed with tap water, counterstained with hematoxylin, and mounted in synthetic mount.

Enzyme-linked immunosorbent assay

For MoAbs screening or specificity evaluation using various parasites antigens, enzyme linked immunosorbent assay (ELISA) was performed as follows:

Flat-bottomed microtitre plates (Greiner, Frickenhausen, W. Germany) were coated at 50 μl/well with 1 μg/ml somatic antigens of *E. multilocularis* or each parasites in 0.05 M $NaHCO_3$/Na_2CO_3 buffer (pH 9.6) and left overnight at 4°C. The wells were washed three times with PBS containing 0.05% Tween 20 (PBS-Tween), blocked at 100 μl/well with 1% BSA-PBS for 1 hour at room temperature. After 3 times washing with PBS-Tween, 50 μl of each hybridoma culture supernatant were added to each well and incubated for 1 hour at room temperature. Following 4 times washing in PBS-Tween, 50 μl of a 1:2,000 dilution of peroxidase conjugated goat anti-mouse IgG or anti-mouse IgM were added to each well and incubated for 1 hour at room temperature. After 5 times washing in PBS-Tween, 100 μl of substrate solution containing 0.04% o-phenylene diamine and 0.007% H_2O_2 in citrate-phosphate buffer (pH 5.0) were added to each well and incubated for 15 min at 37°C. The reaction was then stopped by the addition of 50 μl of 4 N H_2SO_4 and the plates were read at OD 490 nm.

SDS-PAGE and Immunoblotting

SDS-PAGE was performed on 10% acrylamide slabs according to the method of Laemmli (1970) using a AE-6450 apparatus (ATTO, Tokyo, Japan). Mol. wt. markers were purchased from BioRad Laboratories.

After SDS-PAGE, the unstained gel was equilibrated with the transfer buffer (100 mM Tris, 192 mM Glycine, 20% Methanol). Resolved proteins

were transferred electrophoretically to a polyvinylidene difluoride microporous membrane (Immobilon™ PVDF, MILLIPORE, Bedford, USA) using a AE-6670 apparatus (ATTO) at 4°C overnight (100 mA Constant Current). PVDF strip transferred marker proteins was cut from PVDF sheet and stained with amido black. The remainder of the sheet was cut into strips and each strip was blocked with 3% skim milk in PBS overnight at 4°C. After washing with PBS-Tween, PVDF strips were incubated with hybridoma culture supernatant for 1 hour at room temperature. The PVDF strips were washed with PBS-Tween 4 times at 5 min intervals and subsequently incubated with peroxidase conjugated goat anti-mouse IgG or anti-mouse IgM, diluted at 1:1,000 in PBS-Tween containing 0.5% BSA and 0.5% casein, for 1 hour at room temperature. After 5 times washing as described above, PVDF strips were incubated with substrate solution containing 0.05% DAB and 0.01% H_2O_2 in Tris-HCl buffer (pH 7.6) for 10–30 min until the protein bands were visualized. Then the strips were sufficiently washed with tap water to stop the reaction.

Experimental infection of dogs and coproantigen detection

E. multilocularis protoscoleces were maintained in our laboratory using Mongolian gerbils. The origin and methods used for maintenance have been described by Kamiya and Sato (1990). Protoscoleces were prepared from hydatid cysts from a gerbil that had been infected by i.p. injection of larval tissue of the cestode 4 months before.

Three helminth-free dogs were dosed with approximately 200,000 protoscoleces orally. Feces from these dogs were collected before infection and everyday postinfection until autopsy on day 20 PI. Coproantigen was basically prepared as described by Wedrychowicz *et al.* (1983). Briefly, collected feces were preserved overnight at 4°C and then 5 g of feces was mixed with 20 ml of PBS (pH 7.2). Fecal solution was filtered using a mesh and stirred for 24 hours at 4°C. The solution was centrifuged at 6,000 g for 30 min and supernatant was used for coproantigen detection. For coproantigen detection, sandwich ELISA was performed as follows; flat-bottomed microtitre plates were coated at 50 μl/well with 1 μg/ml rabbit anti-*E. multilocularis* somatic antigen antibodies in 0.05 m $NaHCO_3/Na_2CO_3$ buffer (pH 9.6) and left overnight at 4°C. The wells were washed three times with PBS-Tween, blocked at 100 μl/well with 1% BSA-PBS for 1 hour at room temperature. After 3 times washing with PBS-Tween, 50 μl of feces solution was added to each well and incubated for 2 hours at room temperature. Following 4 times washing in PBS-Tween, 50 μl of EmA9 hybridoma culture supernatant were added to each well and incubated for 1 hour at room temperature. The rest of the procedures were the same as previously described i.e. ELISA, however HRP conjugated rabbit anti-mouse IgG+IgM+IgA (Zymed Laboratories, San Francisco, USA) at a dilution of 1:1,000 was used to visualize the reaction.

Results

MoAbs

Eleven MoAbs were obtained after cell fusion. Six of them were IgM, designated as EmA1, EmA2, EmA3, EmA4, EmA5 and EmA6. Five were IgG, designated as EmA7, EmA8, EmA9, EmA10 and EmA11.

Immunoperoxidase staining of histological section

The staining pattern using eleven MoAbs was shown in Table 1. Eleven MoAbs were classified to 7 types by the tissue staining pattern. EmA1-EmA6 all reacted with the testis; besides, EmA1 and EmA2 showed the similar staining pattern reacting with the uterus, eggs, parenchyma, vagina, cirrus and cirrus sac (Type I). EmA3 and EmA6 reacted with the vagina, cirrus, tegument of scolex, sucker and rostellum in addition to reacting with the testis, but not reacted with the tegument of proglottids (Type II). EmA4 strongly reacted with the testis, ciruus and vas deferens (Type III). EmA5 reacted with the intestine of dogs and the whole worm section except the tegument, thus quite different from other MoAbs (Type IV). EmA7 no reacted with any (Type O). EmA8, EmA9 and EmA11 reacted with the parenchyma and tegument showing similar staining pattern (Type V). EmA10 reacted with the eggs, uterus, vagina, carcareous corpuscles, and slightly reacted with the testis (Type VI). In addition, EmA3, EmA6, EmA8, EmA9 and EmA11 reacted with the host intestinal epithelium around the worm. IgM MoAbs,

Table 1 The reaction sites of the worm by IP using eleven MoAbs

	Monoclonal antibodies										
reactive sites	EmA1	EmA2	EmA3	EmA4	EmA5	EmA6	EmA7	EmA8	EmA9	EmA10	EmA11
rostellum			+		+	+					
sucker			+		+	+					
tegument											
scolex			+			+		+	+		
proglottids								+	+		+
parenchyma	+	+	+		+	+		+	+		+
carcareous corpuscles	+	+			+	+		+	+	+	+
excretory canal									+		
ovary					+						
vitelline gland					+						
uterus	+	+			+					+	
egg	+	+			+					+	
vagina	+	+	+			+				+	
testis											
immature					+	+					
mature	+	+	+	+	+	+				+	
cirrus	+	+	+	+		+				+	
vas deferens				+							
host intestinal epithelium around the worm			+		*	+		+	+		+

*EmA5 reacted with the whole intestinal tissue of the host.

EmA1-EmA6, strongly reacted with the testis, while IgG MoAbs, EmA7-EmA11, scarcely reacted with it.

Reaction of MoAbs with parasites antigens

ELISA results using various parasites antigens are shown in Fig. 1. In addition to the antigen of *E. multilocularis* adult worms, each MoAbs reacted with other parasite antigens as follows: EmA1 and EmA2 showed similar reaction pattern, i.e. reacted with the antigens of *T. hydatigena* and *T. taeniaeformis* adult worms. EmA3 and EmA6 reacted with the antigens of *E. multilocularis* protoscoleces, *T. hydatigena, Spirometra erinaceieuropaei* and *Toxocara canis* adult worms. In particular, these MoAbs strongly reacted with the antigens of *S. erinaceieuropaei* and *T. canis*. EmA4 and EmA5 reacted with all parasites antigens used in ELISA. All IgG antibodies, EmA7-EmA11, did not react with the antigens of *S. erinaceieuropaei* and *T. canis* adult worms. EmA9 reacted with the antigen of *E. multilocularis* adult worms only.

Immunoblotting

Recognition pattern in immunoblotting is shown in Fig. 2. IgM MoAbs showed some bands except EmA5. IgG MoAbs also showed some bands. EmA8, EmA9 and EmA11 showed a similar recognition pattern, i.e. showed a smear in a broad molecular weight range of 20–100kD. Both IgM and IgG antibodies from normal mice sera showed no band (not shown).

Coproantigen detection

The result of coproantigen detection in feces of experimentally infected dogs is shown in Fig. 3. In all dogs, coproantigens were detected as early as 3 days PI and continued to be positive until autopsy on day 20 PI. Cut off value was calculated as the mean value +3SD using fecal samples of the 3 dogs before infection. The number of infected worms in each dog was not counted.

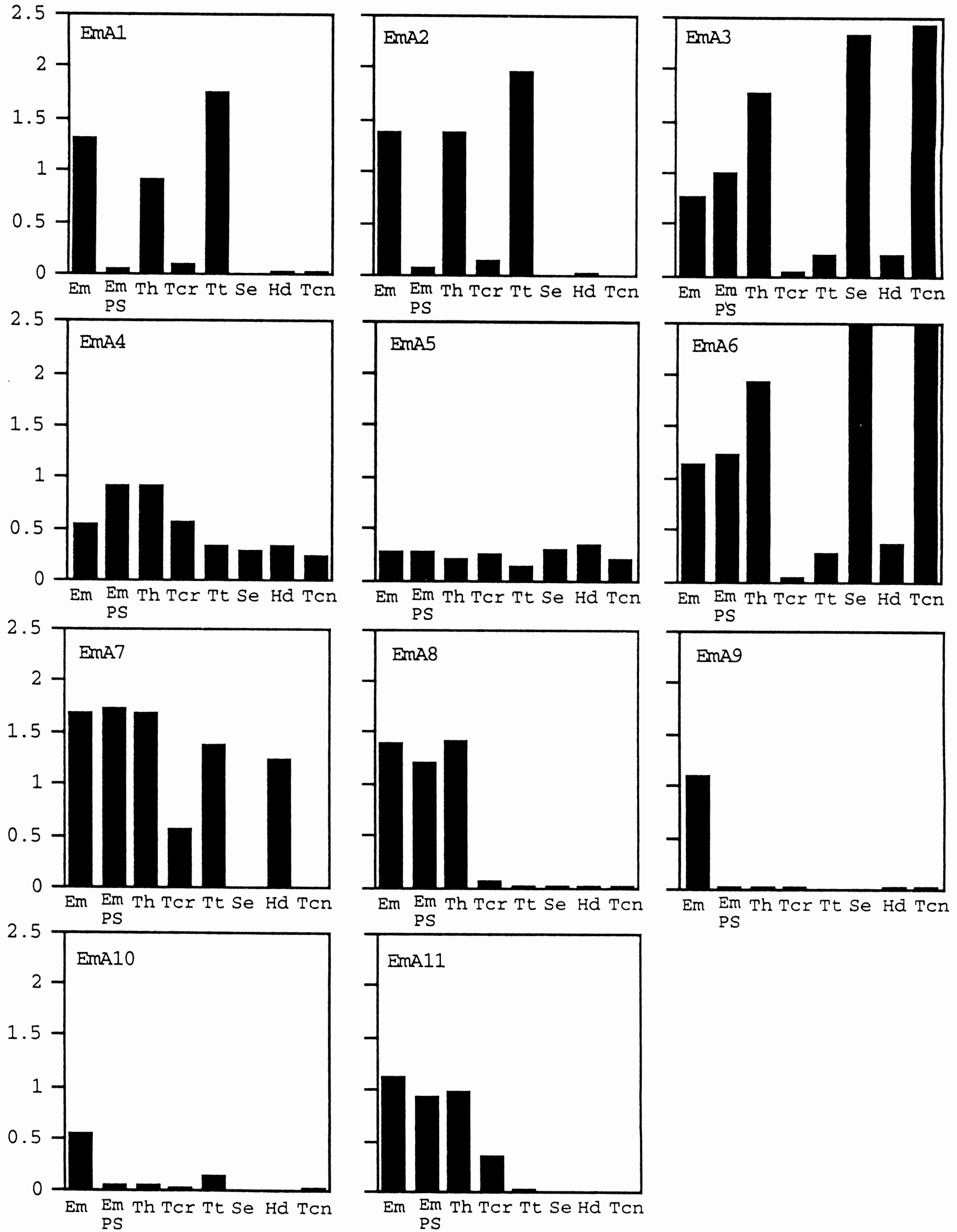

Fig. 1 Specificity evaluation of monoclonal antibodies with various parasite antigens. Em, somatic antigen of *Echinococcus multilocularis* adult worms; EmPS, *E. multilocularis* protoscoleces; Th, *Taenia hydatigena*; Tcr, *T. crassiceps*; Tt, *T. taeniaeformis*; Se, *Spirometra erinaceieuropaei*, Hd, *Hymenolepis diminuta*; Tcn, *Toxocara canis*.

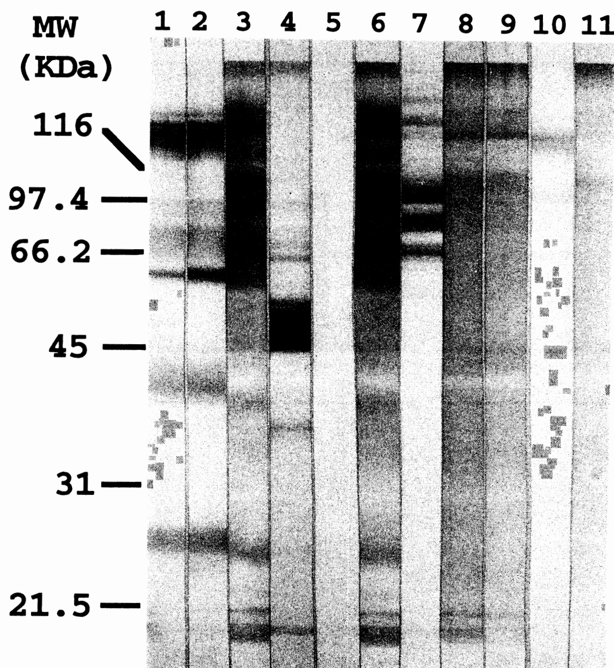

Fig. 2 Antigen recognition patterns of MoAbs in immunoblotting.
Lanes 1–6: IgM MoAbs, EmA1–EmA6.
Lanes 7–11: IgG MoAbs, EmA7–EmA11.

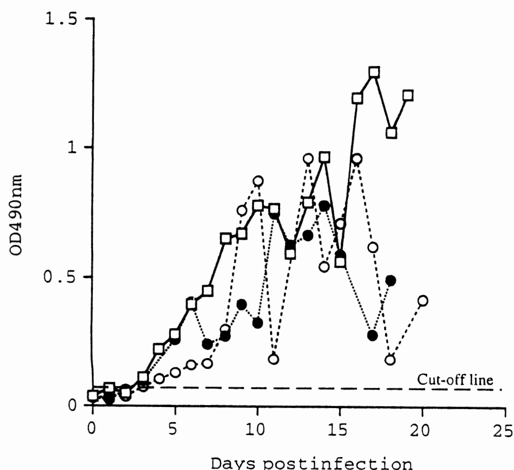

Fig. 3 Detection of coproantigens using EmA9 in feces of experimentally infected dogs. Dog No. 1 (□), Dog No. 2 (●), Dog No. 3 (○). The cut-off line was determined as described in the text.

Discussion

In the present study we have attempted to develop MoAbs for immunodiagnosis of *E. multilocularis* infected definitive hosts. Eleven MoAbs were produced and examined by immunoperoxidase tissue staining (IP) and ELISA. The MoAbs were classified to seven types by the tissue staining pattern. Type O (EmA7) did not react in spite of high antibody titre detected in ELISA. In the present study we used alcohol for dehydration and xylene for penetration. Soluble substances during this process, i.e. lipid or glycolipid, might be deleted in the section or lost their antigenicity. Type II and V reacted with the host intestinal epithelium around the worm and the tegument of scolex and/or proglottids. Furthermore, EmA9 in Type V specially showed reactivity with the excretory canal of the worm. Thus, these MoAbs may recognize E/S products of the worm. Regarding the immuno-

diagnostic use of MoAbs, Type V MoAbs could be useful for the detection of coproantigens or circulating antigens because of their reactivity with E/S products of the worm.

The evaluation of MoAbs using various parasite antigens in ELISA showed that MoAbs have a cross-reactivity to the other parasite antigens. The reactivity pattern of MoAbs in ELISA is correlated to the tissue staining pattern by which MoAbs were classified. However, EmA9, classified as Type V, was distinct from the MoAbs in its class, i.e., no reaction to antigens of *E. multilocularis* protoscoleces and other parasites, thus this MoAb is thought to be specific to antigens of adult *E. multilocularis*. From its specificity and reaction characteristics to the adult worm antigens, EmA9 was considered to be useful for immunodiagnosis of *E. multilocularis* infected definitive host, specially for detection of the E/S antigens in feces, i.e. coproantigens, or in sera. EmA10 only cross-reacted to the antigen of *T. taeniaeformis* that is not found in dogs, but EmA10 mainly recognize genital organs of worm and is not suitable to detect the E/S products of the worm.

To detect the coproantigens, sandwich ELISA using polyclonal antibodies has been used (Allan and Craig, 1989; Allan *et al.*, 1990, 1992; Deplazes *et al.*, 1990, 1991, 1992; Maas *et al.*, 1991, 1992). In the present study, sandwich ELISA using rabbit polyclonal antibodies against somatic antigens of *E. multilocularis* adult worms and monoclonal antibody, i.e. EmA9, was performed to detect coproantigens. In all experimentally infected dogs, the coproantigens were detected as early as 3 days PI. Deplazes *et al.* (1992) used approximately 500,000 protoscoleces for experimental infection of five dogs and coproantigens were not detected on day 5 PI in three dogs out of five. Since we used 200,000 protoscoleces for the infection of all dogs, our test may be more sensitive during the initial phase of infection. EmA9 has a high specificity to the worm antigens, therefore ELISA OD values of fecal samples of three dogs before infection as negative controls were very low and it resulted in a low cut-off value. For evaluating the sensitivity of the test, a large number of controls must be used. Furthermore, cross-reactivity testing using feces of dogs infected with other parasites is necessary for evaluating the specificity of this MoAb.

In immunoblotting analysis, MoAbs detected several bands. The band pattern of antigens detected by MoAbs correlates to the tissue staining pattern by which MoAbs were classified. Based on the results of the MoAb analysis in this study, the following MoAbs are likely the same: EmA1 and EmA2; EmA3 and EmA6; EmA8 and EmA11. EmA5 detected no band in spite of reacting with *E. multilocularis* antigens by IP and ELISA. The antigens recognized by this MoAb may have lost its antigenicity during treatment of SDS and/or 2ME. EmA8, EmA9 and EmA11 reacted with antigenic components in a broad range of molecular weights between 20–100 KDa. This smear band is due to the glycoprotein nature of parasite antigen (Draelants *et al.*, 1995), i.e. these MoAbs may recognize the carbohydrate moiety of the antigen. Since EmA9 recognize the carbohydrate epitope of antigen that is generally heat-stable, it is possible that the fecal samples, e.g. in the case of coproantigen detection test, may be heated to render it safe for handling before the test is performed.

Although further examination is required, the present sandwich ELISA using selected MoAb, designated EmA9, showed a potential to be good tool for immunodiagnosis of *E. multilocularis* infected definitive host.

Acknowledgments

We would like to thank Dr. H. Sato, Department of Parasitology, Hirosaki University School of Medicine and Dr. O. Fujita, Department of Veterinary Science, National Institute of Health, for their valuable help. We would also like to thank Dr. J. A. Abella, Department of Animal Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, for correcting the manuscript.

References

1) Allan, J. C., Avila, G., Garcia, N. J., Flisser, A. and Craig, P. S. (1990): Immunodiagnosis of taeniasis by coproantigen detection. Parasitology, 101, 473–477.
2) Allan, J. C. and Craig, P. S. (1989): Coproantigens in gut tapeworm infections: *Hymenolepis diminuta* in rats. Parasitol. Res., 76, 68–73.
3) Allan, J. C., Craig, P. S., Garcia, N. J., Mencos, F., Liu, D., Wang, Y., Wen, H., Zhou, P., Stringer, R., Rogan, M. and Zeyhle, E. (1992): Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. Parasitology, 104, 347–355.
4) Baumann, D. and Gottstein, B. (1987): A double-anti-

body sandwich ELISA for the detection of *Entamoeba histolytica* antigen in stool samples of humans. Trop. Med. Parasitol., 38, 81–85.

5) Brandt, J. R. A., Geerts, S., De Deken, R., Kumar, V., Ceulemans, F., Brijs, L. and Falla, N. (1992): A monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginata* cysticercosis. Int. J. Parasitol., 22, 471–477.

6) Chamekh, M., Facon, B., Dissous, C., Haque, A. and Capron, A. (1990): Use of a monoclonal antibody specific for a protein epitope of *Echinococcus granulosus* antigen 5 in a competitive antibody radioimmunoassay for diagnosis of hydatid disease. J. Immunol. Methods, 134, 129–137.

7) Craig, P. S., Hocking, R. E., Mitchell, G. F. and Rickard, M. D. (1981): Murine hybridoma-derived antibodies in the processing of antigens for the immunodiagnosis of hydatid (*Echinococcus granulosus*) infection in sheep. Parasitology, 83, 303–317.

8) Craig, P. S., Macpherson, C. and Nelson, G. S. (1986): The identification of eggs of *Echinococcus* by immunofluorescence using a specific anti-oncospheral monoclonal antibody. Am. J. Trop. Med. Hyg., 35, 152–158.

9) De ST. Groth, S. F. and Scheidegger, D. (1980): Production of monoclonal antibodies: Strategy and tactics. J. Immunol. Methods, 35, 1–21.

10) Deplazes, P., Eckert, J., Pawlowski, Z. S., Machowska, L. and Gottstein, B. (1991): An enzyme-linked immunosorbent assay for diagnostic detection of *Taenia saginata* copro-antigens in humans. Trans R. Soc. Trop. Med. Hyg., 85, 391–396.

11) Deplazes, P., Gottstein, B., Eckert, J., Jenkins, D. J., Ewald, D. and Jimenez, P. S. (1992): Detection of *Echinococcus* coproantigens by enzyme-linked immunosorbent assay in dogs, dingoes and foxes. Parasitol. Res., 78, 303–308.

12) Deplazes, P., Gottstein, B., Stingelin, Y. and Eckert, J. (1990): Detection of *Taenia hydatigena* copro-antigens by ELISA in dogs. Vet. Parasitol., 36, 91–103.

13) Draelants, E., Brandt, J. R. A., Kumar, V. and Geerts, S. (1995): Characterization of epitopes on excretory-secretory antigens of *Taenia saginata* metacestodes recognized by monoclonal antibodies with immunodiagnostic potential. Parsite Immunol., 17, 119–126.

14) 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.

15) Gasser, R. B., Lightowlers, M. W., Obendorf, D. L., Jenkins, D. J. and Rickard, M. D. (1988): Evaluation of a serological test system for the diagnosis of natural *Echinococcus granulosus* infection in dogs using *E. granulosus* protoscolex and oncosphere antigens. Aust. Vet. J., 65, 369–373.

16) Gottstein, B., Deplazes, P. and Aubert, M. (1992): *Echinococcus multilocularis*: immunological study on the "Em2-positive" laminated layer during in vitro and in vivo post-oncospheral and larval development. Parasitol. Res., 78, 291–297.

17) Harrison, L. J. S., Joshua, G. W. P., Wright, S. H. and Parkhouse, R. M. E. (1989): Specific detection of circulating surface/secreted glycoproteins of viable cysticerci in *Taenia saginata* cysticercosis. Parasite Immunol., 11, 351–370.

18) Heath, D. D., Lawrence, S. B., Glennie, A. and Twaalfhoven, H. (1985): The use of excretory and secretory antigens of the scolex of *Taenia ovis* for the serodiagnosis of infection in dogs. J. Parasitol., 71, 192–199.

19) Jenkins, D. J. and Rickard, M. D. (1985): Specific antibody responses to *Taenia hydatigena, Taenia pisiformis* and *Echinococcus granulosus* infection in dogs. Aust. Vet. J., 62, 72–78.

20) 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.

21) Kamiya, M. and Sato, H. (1990): Survival, strobilation and sexual maturation of *Echinococcus multilocularis* in the small intestine of golden hamsters. Parasitology, 100, 125–130.

22) Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London), 227, 680–685.

23) Maass, M., Delgado, E. and Knobloch, J. (1991): Detection of *Taenia solium* antigens in merthiolate-form preserved stool samples. Trop. Med. Parasitol., 42, 112–114.

24) Maass, M., Delgado, E. and Knobloch, J. (1992): Isolation of an immunodiagnostic *Taenia solium* coproantigen. Trop. Med. Parasitol., 43, 201–202.

25) Nakane, P. K. and Perse, G. B. JR. (1967): Enzyme-labeled antibodies for the light and electron microscopic localization of tissue antigens. J. Cell Biol., 33, 307-318.

26) Wedrychowicz, H., Maclean, J. M. and Holmes, P. H. (1983): The detection and measurement of coproantibodies to *Nippostrongylus brasiliensis* in rats following a primary infection. Parasite Immunol., 5, 277–287.