



Title	Production of monoclonal antibodies against Echinococcus multilocularis excretory/secretory antigens and its use in coproantigen detection
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small cysts. Histologically, cyst wall was composed of an inner thin germinal layer and an outer thick laminated layer. Inside the germinal layer, brood capsules and protoscoleces were formed and the protoscoleces were observed normally in the brood capsules. External development or protoscoleces on outer surface of brood capsules were partially observed. The strongly PAS-positive laminated layer was enclosed by host-produced tissues, and host reactions were espe-

cially strong around the cysts adhered to the host organs.

When *E. vogeli* cyst homogenate was inoculated into the abdominal cavity of Mongolian gerbils and AKR mice, polycystic hydatid cysts were recovered, which is probably the result of multiple exogenous proliferation of small cysts. There were differences in locations and shapes of cysts and in protoscoleces formation between these animals.

Production of monoclonal antibodies against
Echinococcus multilocularis excretory/secretory antigens
and its use in coproantigen detection

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Nine murine monoclonal antibodies (mAbs) against excretory/secretory (ES) antigens of adult *Echinococcus multilocularis* (EM) were produced. Three of them were IgG₁ (designated as Em a, Em h and Em i) and six were IgM (Em b, Em c, Em d, Em e, Em f and Em g). When the reactivity of mAbs were tested by ELISA against the various parasite antigens including *Echinococcus* and *Taenia* species, three types of reaction were observed (Em a ; Em b-g ; Em h and i). Only Em h and Em i selectively reacted to EM adult somatic and ES antigens and no cross-reactions were observed with *Taenia* species derived antigens including *Taenia hydatigena*. Western blot analysis of mAbs with EM ES antigens showed a smear in a broad molecular weight range. In immunohistochemistry, all of mAbs recognized the antigens of tegument and host intestinal epithelium around the worm. Because those reactions disappeared after periodate oxidation, it is suggested that the

epitope recognized by mAbs are composed of carbohydrates moieties.

Em i was applied to coproantigen detection and sandwich ELISA using Em i and rabbit polyclonal antibodies against EM ES antigens was performed, however, the sensitivity of the ELISA was found to be low. To improve the sensitivity of the coproantigen detection, avidin biotinylated peroxidase complex method was applied. Sandwich ELISA's using rabbit polyclonal antibodies as capture antibody, Em i as primary antibody and biotinylated anti-mouse horse IgG as secondary antibody (rAb / Em i / b-AntiM), and that using Em i as capture antibody and biotinylated mAb, EmA9, which had been produced against EM somatic antigens and used in the present coproantigen detection technique, as primary antibody (Em i / b-EmA9) were found to be appropriate for coproantigen detection.

The selected two methods were compared with current technique (rAb / b-EmA9) using

fecal samples collected from wild foxes. Since the antigenicity did not decrease by heat and formalin treatment, all the fecal samples were treated by heat and formalin to sterilize Em eggs. The rAb / Em i / b-AntiM and rAb / b-EmA9 showed the same detection rate of positive samples. Feces of non-Em infected foxes showed low OD values in all of the three methods, even though the foxes were infected with variety of helminths excluding *Echinococcus* and *Taenia* species. Using the feces obtained

from experimental infections with *T. hydatigena*, *E. granulosus* and *E. vogeli*, specificity of the methods were evaluated. Although rAb / b-EmA9 cross-reacted with *T. hydatigena* coproantigen at the onset of egg excretion, neither rAb / Em i / b-AntiM nor Em i / b-EmA9 reacted with *T. hydatigena* coproantigen. All methods detected coproantigens of *E. granulosus* and *E. vogeli*, suggesting genus specific diagnosis of *Echinococcus* infection can be feasible by the developed methods.

Morphological and molecular genetic analyses of jumbled spine and ribs (*Jsr*) mutant mouse

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Vertebral abnormality derived from genetic mutation has often been observed in many animals including human and mice. Since the vertebral development, beginning with somite formation, is associated with the expression of many genes, mutant mice have been contributed to understand these mechanisms. Jumbled spine and ribs (*Jsr*) mice, showing the irregular segmentation of axial skeleton was found as spontaneous mutation. The previous study revealed that the causal gene of *Jsr* related to the cell growth of somite and sclerotome, and that this abnormality was due to a single autosomal dominant gene. As a result of a high resolution map around *Jsr* with 1,026 backcross progeny generated by mating CKH and MOG, *Jsr* was mapped at the centromeric position with 0.2+/-0.14 cM (centi morgan) from *D5Mit24* and *D5Mit22* locating chromosome 5. In the present study, further morphological and genetic analyses were performed.

It was confirmed by histological observations that axial skeletons showed various abnormalities such as asymmetry, fusion and spina bifida. At 11.5 days of gestation, parasagittal sections of heterozygotes (*Jsr/+*) embryos revealed that the irregular arrangements of primitive vertebra and somite clefts were marked as well as the disappearance of boundary region between primitive vertebra. It was suggested that the causal gene was associated with the development of presomitic mesoderm.

The locus of candidate gene, *Lfng* (lunatic fringe), corresponded completely to that of *Jsr* according to genetic mapping. In the previous report, *Lfng* deficient mice was markedly similar to *Jsr* mutant, showing an phenotype, such as irregular segmentation in embryos at 9.0 days of gestation and entirely shortened and fused vertebrae, in adult mouse. These findings assumed that *Lfng* might be a strong possibility as causal gene of *Jsr*.