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The study on experimental secondary
pulmonary alveolar echinococcosis in rats

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Alveolar echinococcosis (AE), caused by the metacestode of *Echinococcus multilocularis*, is one of the most serious parasitic zoonoses. The primary lesions are mainly established in the liver, but occasionally metastasizes to distant organs including lung. Although pulmonary AE is often observed in humans, there is no report of pulmonary AE in experimental animals. The purpose of this study is to establish animal model of pulmonary AE with rats and to investigate the pathogenetic mechanism of lung lesion. Thirty eight-week-old male Wistar rats were used in this experiment. Each rat was injected intravenously from the tail vein with about 3,000 protoscolexes, and histopathological examination, hematological test, MRI examination and sero-diagnostic test were performed from 1 to 50 weeks post infection(PI).

Macroscopically, a parasitic cyst (about 1 mm in diameter) was first observed 2 weeks PI and 33 rats had lung lesions in the examination. Parasitic cysts increased in both number and size and changed morphologically from unilocular to multilocular appearance during the experiment.

Histologically, the cyst wall consisted of an outer laminated cuticular layer and an inner germinal layer surrounded by granulation tissue and inflammatory cell infiltration with lymphocytes and plasma cells. Nature of granulation tissue is changed during the examination. Protoscolexes were first observed 4 weeks PI. From 9 weeks PI, liquefaction necrosis, slight degree of calcification and organization were observed in some lesions.

Under MRI examination, the gross appearance of the cysts were well correspond with the MR images. Early cystic lesions that had no "hydatid sand" appeared black on T1-weighted image, had equal signal intensity to the skeletal muscle on proton-weighted image and were white on T2-weighted image. Signal intensity of cystic lesions that had "hydatid sand" was similar on proton- and T2-weighted images, but of lower intensity than the skeletal muscle on T1-weighted image. Signal intensity of degenerative lesions such as liquefaction necrosis and fibrosis were low or high depending on the contents. Although all evaluation of the lung lesions was performed on MRI after the euthanasia, imaging under anesthesia was possible and motion artifact could be reduced by synchronizing time of repetition (TR) with respiratory and heart rates.

The antibody (Ab) responses against metacestode were assessed by Western blotting. The Ab-responses against Em16 and Em18, which are specific antigen of protoscolex, were detected after 9 weeks PI. The response against Em18 appeared stronger than that of against Em16 by 27 weeks PI, but after 31 weeks PI, the latter was stronger than the former.

These results suggest that the rat is a highly useful animal model for experimental pulmonary AE. MRI examination plays an important role in the diagnosis of pulmonary AE, because MRI can depict the multilocular cysts and MR signal intensity can reflect the pathological findings. Furthermore, MRI can detect the parasitic cysts in the lung earlier than was possible with sero-

diagnosis. From the results of serodiagnostic test and pathological examination, it suggests that Em18 is a marker of the early stage of

infection, whereas Em16 shows the advanced stage.

A study of the clinical application of adenosine deaminase in dogs

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This study was carried out in order to investigate the suitability of adenosine deaminase (ADA) for clinical diagnosis in dogs.

Initially the serum and plasma levels of ADA in 26 normal dogs were determined and a reference range was established as follows: 4.67 ± 2.15 IU/l and 4.99 ± 2.78 IU/l, respectively. Then, the effect of following possible influential factors on ADA activity namely; (1) blood lysis, (2) storage condition of whole blood, serum and plasma, and (3) circadian rhythm and lunar periodicity (physiologic variation), were investigated.

It was observed that blood lysis and physiologic variation had no influence on the ADA activity. However, the serum ADA activity in whole blood kept at 25°C increased as the duration of time increased, but this phenomenon was not seen in blood of cows and cats. These results therefore, suggest that keeping whole blood at room temperature for a long time can influence ADA activity in dogs. Furthermore, the ADA activity in serum and plasma remained stable four weeks at -20°C but decreased to half its original level after 12 and 3 hours at 4°C and

25°C, respectively.

To clarify the relationship between lymphocytes and ADA, dog peripheral blood lymphocytes were exposed to ConA in culture. The level of ADA activity in stimulated lymphocytes increased about 2.5 times more than that of controls. Furthermore, the toxic effect of 2-Chlorodeoxyadenosine (CdA), one of ADA inhibitors commonly used in humans, to dog peripheral blood lymphocyte activation was investigated during induction by ConA. CdA did not inhibit lymphocytes activation.

The clinical significance of adenosine deaminase was tested in three cases of canine lymphoma. Serum ADA levels were measured following chemotherapy. It could not make clearly correlate ADA activity with tumor behavior.

Finally, this study provides fundamental data on ADA activity in whole blood, plasma and serum of dogs under different storage conditions. It further provides information on changes in ADA activity in ConA stimulated lymphocyte. This data will therefore, be useful in future studies investigating the clinical application of ADA in dogs.