

{ Poster Presentation }

P#1

Expression Patterns of Tumor Necrosis Factor Receptors on Lymphoma Cells in Enzootic Bovine Leukosis

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Tumor necrosis factor-alpha (TNF- α) has been reported to be associated with the progression of lymphoproliferative neoplastic diseases and retroviral infections. Hence we examined immunohistochemically the expression patterns of TNF-receptors (TNF-RI and RII)

on lymphoma cells derived from the 29 enzootic bovine leucosis (EBL). Obtained lymphomas in 29 animals with EBL were histopathologically classified into three types: diffuse mixed type (10 cases), diffuse large type (9 cases), and diffuse large cleaved type (10 cases). Immunohistochemically using a monoclonal antibody to a bovine lymphocyte surface antigen, the lymphomas were classified into three phenotypes: B-1a (CD5+/CD11b+), B-1b (CD5-/CD11b+) and B-2 (conventional B) (CD5-/CD11b-). Interestingly, the lymphoma cells in all animals expressed TNF-RII, but not TNF-RI. Although, in EBL, lymphoma cells of which the histopathological and immunological property differs has been formed, the expression patterns of TNF-Rs had the universality in all lymphoma cells. TNF-RII, which induces cell proliferation, was expressed but TNF-RI, which induces cell apoptosis was not expressed on all lymphoma cells, suggesting that TNF-Rs plays an important role in the malignant proliferation of B cells and formation of lymphomas in EBL.

P#2

Pathology of Neospora Caninum Infection in an Adult Dog

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Introduction

Neospora caninum (*N. caninum*) infection is a relatively new disease that was found from a young dog in 1988 [Dubey et al.,1988], and after that, many cases in calves were reported. The disease develops stillbirth/abortion in neonatal or young calves [Okada et al.,1994,1995 & 2000] and meningoencephalitis and myositis in young dogs [Dubey & Lindsay,1996; Itoh & Uchida,2001; Umemura et al.,1992]. In Japan, there were two reports that occurred in young dogs [Itoh & Uchida,2001; Umemura et al.,1992].

Recently, we met a 7 year-old-dog that developed progressive paralysis in the hindquarters. The disease was diagnosed as *N. caninum* infection by pathological and immunohistochemical investigation and gene analysis.

Material and methods

Seven-year-old, male Golden Retriever developed bilateral hind paralysis suddenly. The veterinarian(one of the co-workers) made suspicious diagnosis of spinal compression disease, but the compressive change was not detected by X-ray and CT examination. Paralytic condition continued for three months progressively and the dog died finally.

Necropsy of the patient was performed and tissue samples were investigated histopathologically and immunohistochemically (IHC). Used sera for sABC method of IHC were anti-*N. caninum* polyclonal antibody(Ab; VMRD Inc.), anti-*N. caninum* (gp65) monoclonal Ab(VMRD Inc.), and

anti-Toxoplasma gondii Ab(VMRD Inc.) for the differential diagnosis. Electron microscopic observation of the paraffin-embedded samples from the cerebellum and brainstem was done.

Gene analysis of nested polymerase chain reaction (PCR) using two primer sets of *N. caninum* (GenBank through the accession number X84238)[Baszler et al.,1999; Yamage et al.,1996] and subsequent sequencing (Big Dye terminator cycle sequencing kit, Applied Biosystems) for the obtained DNA product was performed.

Results

Compressive changes in the spinal cord was found, but yellow-whitish color changes were recognized in the white matter of the cervical spinal cords macroscopically

Multiple foci of non-purulent meningoencephalomyelitis were formed widely in the brain, cerebellum, brainstem, and spinal cord histologically. Many lesions distributed in the cerebellum and brainstem especially. Main changes were infiltration of round cells and astrogliosis, and secondary demyelination in the spinal cords. Cystic structures that contain many minute parasites inside were scattered in/out of the lesions. In the other organs, nonpurulent myositis with some cysts was observed in ocular muscles. Fine morphology of *Eimeria* sp. tachyzoites observed in the cysts electron microscopically[Speer et al.,1999]. By IHC, the parasites in the cysts were clearly positive for anti-*N. caninum* but were negative for anti-*T. gondii*.

By gene analysis of nested PCR, the specific band that relates to *N.caninum* was detected on the agarose gel. Base composition of the DNA products resulted in 98.2% homology with that of *N.caninum* in the

GenBank. From these serial results the parasite was identified as *N.caninum*.

Discussion

Neosporosis occurs rarely in adult dogs [Lorenzo & Pumarola,2002; Cantile & Arispici, 2002; Greig et al.,1995] and the present case is the first occurrence in an adult dog in Japan. The occurrence at 7 year-old-age made clinical diagnosis of neosporosis difficult, even if recurrence by the treatment was unexpected. In addition, no etiological information, such as the contact with another animals or occurrence of resembling neurologic diseases in the neighborhood was obtained. In the present study, neosporosis was suspected histopathologically and its differential diagnosis was completed by IHC and gene analysis.

Multiple granulomatous myositis seemed to be one of the characteristics of neosporosis as well as meningoencephalitis[Dubey & Lindsay,1996; Itoh & Uchida,2001; Umemura et al.,1992]. In the present case, the same lesions were observed also in the ocular muscles. Other muscles were not observed unfortunately because of poor information about neosporosis, there was a possibility to make clinical diagnosis if muscle biopsy was performed.

P#3

The Histopathogenesis of Paralytic Rabies in Six-week-old C57BL/6J Mice Following Inoculation of the CVS-11 Strain into the Right Triceps Surae Muscle

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Rabies is an ancient disease that is still endemic in many parts of the world. Rabies virus is a highly neurotropic virus that causes fatal encephalomyelitis in humans and animals; however, the precise histopathogenesis including invasion routes from the periphery to the central nervous system (CNS) are not known.

In this study, fixed CVS-11 strain was inoculated into the cerebrum and peripheral muscles of adult C57BL/6J mice, and the primary target cells and the sequential involvement of major regions during infection in peripheral tissues and CNS were compared. A fatal encephalomyelitis developed after intracerebral and hind limb inoculation of six-week-old C57BL/6J mice with fixed rabies virus (CVS-11 strain). With intracerebral inoculation, virus antigens were first detected in the cerebral cortex and hippocampus at two days post-inoculation (PI), and virus later spread centrifugally to the thalamus, brain stem, cerebellum, spinal cord and spinal ganglia. At four days PI, strong morphologic changes of apoptosis and DNA fragmentation were particularly evident in the hippocampus and cerebral cortex. All infected mice died without limb paralysis at 10 to 11 days PI.