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INFORMATION

Hokkaido University conferred the degree of Doctor of Philosophy (Ph.D) in Veterinary Medicine on December 25, 2001 to 2 recipients.

The titles of their theses and other information are as follows :

Phylogenetic and virulence analysis of tick-borne encephalitis viruses in Japan and Russia

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In this study, TBE virus isolation was performed in several regions of east-Siberian and far-eastern Russia, the virus isolates were classified into subtype, and subjected for phylogenetic and evolutionary analysis. The studies were also extended to characterize the pathogenic potential and antigenicity of the isolates. Efficacy of an available TBE vaccine against the TBE virus isolates was also evaluated in mouse model.

First, in order to identify when TBE viruses emerged in Hokkaido, the times of divergence of Oshima and far-east Russia strains were estimated. TBE virus strains were isolated from ticks (*Ixodes persulcatus*) collected in Khabarovsk in 1998 and RNA sequences of the viral envelope protein gene were determined. Based on the RNA sequences, the synonymous substitution rate of these TBE viruses was estimated to be 2.9×10^{-4} per site per year. From the rate and synonymous distance in the phylogenetic tree, the lineage divergence time was predicted to be about 260-430 years ago. It provides the evidence that the TBE virus Hokkaido (Oshima) strains emerged from the far-eastern Russia a few hundred years ago.

Next, TBE virus strains were isolated from east-Siberian (Irkutsk) and far-eastern

region of Russia (Vladivostok and Khabarovsk) in 1998 to 1999. Phylogenetic analysis demonstrated that different subtype strains distribute in Irkutsk (Siberian subtype) and far-eastern region (far-eastern subtype). Although, Irkutsk and far-eastern strains possess similar antigenicity as determined by cross-neutralization test, Irkutsk strains showed a minor difference of antigenicity as revealed by monoclonal antibodies reactivities. Irkutsk strains had a single amino acid change at a position 234 in the envelope protein and this amino acid was considered as a "signature" of Siberian subtype TBE viruses.

The virulence (neuroinvasiveness and neurovirulence) of TBE virus strains isolated in various region were compared by subcutaneous and intracerebral inoculation to mice. As the result, Hokkaido and far-eastern Russia (Oshima, Vladivostok and Khabarovsk) strains (classified as far-eastern subtype) showed almost similar virulence in mice. However, Irkutsk strains showed somewhat higher virulence to mice as compared with Oshima, Vladivostok and Khabarovsk strains. In addition, the LD₅₀ value of Irkutsk strain was about 10-40 fold lower than Vladivostok and Khabarovsk strains. Therefore, it was considered that TBE viruses distributed in

Irkutsk may possess somewhat higher virulence to mice as compared with TBE viruses distributed in far-eastern region.

To establish the preventive vaccination for TBE in east-Siberian and far-eastern regions of Russia, the efficacy of the European TBE vaccine against recent Irkutsk, Vladivostok and Khabarovsk isolates was evaluated. The sera of vaccinated humans showed efficient neutralizing antibody titers against Irkutsk, Vladivostok and Khabarovsk isolates.

In addition, all vaccinated mice were asymptomatic and survived against lethal challenge of each virus strain. These results demonstrated that the European vaccine could induce efficient neutralizing antibodies in humans and protective immune responses in animal model against TBE viruses in east-Siberian and far-eastern regions. Therefore, it was expected that the European vaccine can prevent the TBE virus infection in human in east-Siberian and far-eastern regions.

Original papers of this thesis appeared in "J. Gen. Virol." Vol. 80 : 3127-3135 (1999), "J. Gen. Virol." Vol. 82 : 1319-1328 (2001) and "Vaccine" Vol. 19 : 4774-4779 (2001)

Genetic and antigenic analyses of porcine enteroviruses

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Genetic and antigenic characters of porcine enterovirus serotype 1 (PEV-1) reference strain, Talfan, were analyzed. Furthermore, genetic reclassification of other members of PEVs was performed.

First, the majority of the genomic sequence of Talfan was determined and it was compared with other picornaviruses. Genome structure of Talfan was characteristic in possessing some features; (1) a leader protein coding region at 5' end of the open reading frame, (2) 2A region coding an foot-and-mouth disease-like protease, and (3) putative poly (C) tract in the 5' non-translated region. Moreover, the amino acid identities (a. a. i.) among them was significantly low throughout the genome except 2A protein. Even in 3D region coding RNA-dependent RNA polymerase (RdRp) which is considered to be the

most conserved protein among picornaviruses, the a. a. i. were less than 40%, although it possessed picornavirus characteristics. Phylogenetic analysis of RdRp revealed that Talfan fell into a distinct cluster within picornaviruses. Based on these observation, I proposed that PEV-1 Talfan be regarded as the prototype of a new genus for the family *Picornaviridae*. Consequently, the new genus *Teschovirus* was approved in the eleventh International Congress of Virology in 1999.

Second, antigenic structures of "porcine teschovirus (PTV; renamed from PEV-1)" Talfan strain was analyzed. Neutralizing antigenic sites on Talfan were identified through epitope mapping of neutralizing monoclonal antibodies (MAbs) by using synthetic peptides spanning the capsid proteins. All of the 11 MAbs obtained recognized peptides in the EF