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Establishment of monoclonal antibodies against Borna disease virus p40 protein and the application to a serological diagnostic method

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Borna disease causes polioencephalomyelitis of horses and sheep. The disease has been known to be endemic in Central Europe. Recently, seroepidemiological survey of the causative agent (Borna disease virus, BDV) suggests that the virus distributes in wide areas among different species of animals and is also suspected to be associated with human psychiatric disorders. It is important to search the reservoir animal, however there is no standard serological diagnostic method for BDV infection and the reservoir is not determined yet. Thus, in this study monoclonal antibodies (MAbs) against BDV p40 protein were used to develop capture enzyme linked immunosorbent assay (capture ELISA) which is simple and highly specific serological diagnostic method. Using the capture ELISA, sera from *Rattus norvegicus* were examined for anti-BDV antibody.

1. Eight hybridomas producing MAbs against p40

protein of BDV were obtained using recombinant p40 protein as the immunogen. These MAbs were confirmed to be reactive to native p40 protein by Immunofluorescent antibody assay (IFA) using MDCK cells persistently infected with BDV.

2. The MAbs were separated into three groups from reactivity to p40 in Western blot analysis. B9 and E7 of group 1 can detect the virus antigen in persistently infected rat brain. These two MAbs recognized different epitopes.

3. Sera of low dilution can be tested in the capture ELISA with high specificity and with the same sensitivity as that of the IFA test. This method can detect antibody with low titer and process many samples.

4. Sera from 106 field rats were examined by capture ELISA, but specific antibody to BDV was not detected.

Phylogenetic analysis and transmission cycle of tick-borne encephalitis virus isolated in Hokkaido

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Tick-borne encephalitis virus Oshima-5-10 was isolated from a blood of sentinel dog at Kamiiso, Hokkaido in 1995. Nucleotide sequence

of envelope (E) protein gene of Oshima-5-10 was determined and phylogenetic analysis was performed among Oshima-5-10 and TBE strains in