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

Kouji Tsujimura
Laboratory of Public Health, Department of Environmental Veterinary Sciences, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

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 immunosorbant 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

Daisuke Hayasaka
Laboratory of Public Health, Department of Environmental Veterinary Sciences, School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

Tick-borne encephalitis virus Oshima-5-10 was isolated from a blood of sentinel dog at Kamiiso, Hokkaido in 1995. Nucleotid 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
other countries. Furthermore, virus isolations were conducted from ticks and rodents in the study area.

Results are summarized as follows.
1. Oshima-5-10 showed high degree of identity with Russian spring summer encephalitis (RSSE) virus strain Sofjin; 95.7% in nucleotide sequence and 99.0% in amino acid. Phylogenetic analysis identified Oshima-5-10 as RSSE type virus.
2. Two virus strains were isolated from 600 I. ovatus ticks collected in April and May, 1996. The minimum field infection rate of I. ovatus was calculated as 0.33%(2/600).
3. Virus strains were isolated from A. speciosus trapped in 1995 and C. rufocanus trapped in 1996.
4. Virus isolates from I. ovatus, A. speciosus and C. rufocanus, were identified as TBE virus by indirect immunofluorescent antibody test using monoclonal antibodies. Furthermore, these virus isolates were identified as RSSE type virus by phylogenetic analysis of the E protein genes of each isolates.
5. The sequence size of 3'-noncoding region (3'NCR) of Oshima-5-10 was different from RSSE virus strain Sofjin.

Analysis of immune response and pathogenesis in mice infected with hantavirus

Eiichi Yokota
Laboratory of Public Health
Department of Environmental Veterinary Sciences
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060-0818, Japan

Hantavirus is maintained various murine groups as a reservoir in the nature, and humans become hantavirus infection by contacting with the infected animal. A part of the infected animals holds a virus for a long time, and becomes the persistently infected animals which excrete the virus in the discharges. Therefore, these persistently infected animals is very important as not only the maintenance of the virus in the murine groups, but also the animals which transmit this virus to humans. However, all infected animals don't became persistent infection, and it seems that various factors is related to establish the persistent infection of this virus. So in this thesis, two viral strains which differ from pathogenesity were inoculated to the suckling mice and the adult mice, and it was examined how influence the establish of the persistent infection by a degree of maturity of the immune system and a difference in the viral strains. Further, the preparation of the antigen presentation cell was tried for the measurement of cytotoxic activity to viral antigen.

The results were summarized as follows.
1. In the inoculated adult BALB/c mice, the virus wasn't detected in 4 and 8 weeks after infection and then it seems that the virus was excluded from mice. On the other hand, in the inoculated suckling BALB/c mice, the virus was detected until 8 weeks after infection. These result showed that suckling BALB/c mice inoculated with hantavirus was the model of persistent infection.
2. In the inoculated both suckling mice and adult mice, maximum level of IgG and neutralizing antibody was detected in 4 to 8 weeks after infection, and kept until 12 weeks after infection. On the other hand, IgM antibody was detected in