Production and characterization of monoclonal antibodies against tick-borne encephalitis virus Hokkaido strain

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Tick-borne encephalitis (TBE) patient was found at Kamiiso, Hokkaido in 1993, and a TBE virus (Oshima5–10) was isolated from blood of a sentinel dog in 1995.

To develop a diagnostic reagent to identify TBE virus, monoclonal antibodies (MAbs) were produced against TBE virus Oshima5–10 strain. Reaction patterns of MAbs were examined against several flaviviruses by indirect fluorescent antibody (IFA) test, hemagglutination-inhibition (HI) test, and neutralization (NT) test.

Results were summarized as follows:

1. Seven hybridomas producing MAbs against TBE virus Oshima5–10 strain were obtained using inactivated virus by formaldehyde as the immunogen. These Mabs were comfirmed to react with viral protein by IFA test.
2. All MAbs were comfirmed to react with envelope protein of Oshima5–10 strain by Western blot analysis after immunoprecipitation.
3. Two MAbs 4H8 and 4A2 showed high antibody titer to 4 strains of tick-borne encephalitis virus group and Japanese encephalitis virus by IFA test or HI test, which suggests 4H8 and 4A2 to be flavivirus genus-specific.
4. MAb 1H4 showed high antibody titer to 4 strains of tick-borne encephalitis virus group by IFA, HI and NT tests, suggesting 1H4 to be TBE group-specific.
5. MAb 2F9 showed high antibody titer to Sofjin strain and Oshima5–10 strain, suggesting 2F9 to be Russian spring-summer encephalitis type-specific.
6. The other MAbs, 2A5, 4H1 and 5D10 did not show consistant reaction patterns to each virus strain.

Development of highly sensitive hantavirus genome detection methods and analysis of the viral replication

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To analyze the Hantavirus replication cycle of plus-strand and minus-strand RNA in infected Vero E6 cells and newborn rats, we developed a Northern blot hybridization technique (NB) using digoxigenin labeled RNA probes to quantitatively analyse the Hantavirus genome and develop a highly sensitive and strand specific RT-nested-PCR (SS-PCR).

The results are summarized as follows:

1. NB and SS-PCR could specifically detect either