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Author(s)	TANABE, Hiroki
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Production and characterization of monoclonal antibodies against tick-borne encephalitis virus Hokkaido strain

Kimiyo Komoro

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

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

Hiroki Tanabe

Laboratory of Public Health,
Department of Environmental Veterinary Sciences,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo, Japan

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

plus- or minus- strand hantavirus S genome RNA in which the sensitivities of NB and SS-PCR were 107 and 103 or more viral RNA molecules, respectively.

2. In Vero E6 cells infected with KI-83-262 (KI) strain of Seoul type hantavirus, plus-stranded RNA of the S genome began to synthesize immediately after inoculation followed by minus-strand RNA synthesis, and then infectious virus particles were released into the media from 24 hours after inoculation.

3. Newborn rats were inoculated with KI strain through three different inoculation routes[subcutaneous (s.c.), intranasal (i.n.), per os (p.o.)]. Only s.c. inoculated rats exhibited both plus- and minus-strand virus genome RNA. It was revealed that the s.c. route was an efficient way to induce infection for viral RNA replication in rats.

4. In the s.c. inoculated rats, both plus-strand and minus-strand virus RNA was detected at 1 week after infection, but, only plus-strand virus RNA was detected at 1 month after infection. This indicates that plus-stranded RNA may be predominant in persistently infected animals.

5. Hantavirus genome RNA was detected by SS-PCR not only in s.c. rats but also in i.n. and p.o. rats at 1 week and 1month after infection. Therefore, even in rats infected through less efficient routes, once the infection is established, extremely small amounts of hantavirus RNA can be persistently sustained.

Photosensitizer, pheophorbide *a*, induces caspase-3-dependent apoptosis in Chinese hamster V79 fibroblast cells.

Asuka YOSHITO

Laboratory of Radiation Biology,
Department of Environmental Veterinary Sciences,
School of Veterinary Medicine,
Hokkaido University, Sapporo 060–0818, Japan

Pheophorbide *a* (PPa) is a haematoporphyrin derivative, which is a causal substance of dietary photosensitization in animals. The illumination of haematoporphyrin derivatives such as PPa with visible light is known to produce singlet oxygen ($^1O_2$) and to induce cell death, which is called photodynamic effects. Therefore, haematoporphyrin derivatives are sometimes utilized for the tumor therapy as a photosensitizer. Recently, it was reported that the apoptotic pathway caused a considerable part of photodynamic-induced cell death. Using Chinese hamster V79 fibroblast cells treated with PPa and visible light, the present study was carried out to clarify whether the apoptotic signaling pathway from the release of cytochrome c (Apaf-2) from mitochondria to the activation of caspase-9 (Apaf-3) and caspase-3 (CPP32) was involved in photodynamic-induced cell death.

Flow cytometric analysis combined with DNA end-labeling technique using FITC-conjugated-dUTP (TUNEL method) showed that apoptotic cell was induced after exposure of Chinese hamster V79 cells to visible red light (580～700nm) in the presence of PPa and the number of apoptotic cells time-dependently increased. The induction of apoptosis was further confirmed by the appearance of ladder-like DNA fragmentation after the PPa-treatment. Western blot analysis of S-100 fraction of V79 cells revealed that cytochrome c (Apaf-2) was released from mitochondria by the PPa-treatment.