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VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 13, Number 6, 2013

DOI: 10.1089/vbz.2012.1231

Genetic and Biological Characterization of Tick-Borne Encephalitis Virus Isolated from Wild Rodents in Southern Hokkaido, Japan in 2008

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Abstract

Tick-borne encephalitis virus (TBEV) is a zoonotic agent causing severe encephalitis in humans. A recent epizootiological survey indicated that endemic foci of TBEV have been maintained in the southern part of Hokkaido until recently. In this study, we sought to isolate TBEV from wild rodents in the area. One virus, designated Oshima 08-As, was isolated from an *Apodemus speciosus* captured in Hokuto in 2008. Oshima 08-As was classified as the Far Eastern subtype of TBEV and formed a cluster with the other strains isolated in Hokkaido from 1995 to 1996. Thirty-six nucleotide differences resulted in 12 amino acid changes between Oshima 08-As and Oshima 5–10 isolated in 1995. Oshima 08-As caused high mortality and morbidity in a mouse model compared with Oshima 5–10. Although similar transient viral multiplication in the spleen was observed in the mice infected with Oshima 08-As and Oshima 5–10, greater viral multiplication with an inflammatory response was noted in the brains of mice infected with Oshima 08-As than those infected with Oshima 5–10. These data indicate that a few naturally occurring mutations affect the pathogenicity of the Oshima strains endemic in the southern part of Hokkaido.

Key Words: Tick-borne encephalitis virus—Oshima 08-As—Oshima 5–10—Hokkaido.

Introduction

TICK-BORNE ENCEPHALITIS VIRUS (TBEV), a member of the genus *Flavivirus* within the family Flaviviridae, causes tick-borne encephalitis (TBE) in humans. Although most of the cases are asymptomatic, TBEV produces a variety of clinical symptoms. TBEV is prevalent over a wide area of Eurasia, including Europe, Russia, Far Eastern Asia, and Japan (Blaskovic et al. 1967, Korenberg and Kovalevskii 1999, Lindgren and Gustafson 2001) and has a significant impact on public health in these endemic regions. On the basis of phylogenetic analysis, TBEV can be divided into three subtypes—the Far Eastern subtype, known as Russian Spring summer encephalitis (RSSE) virus; the European subtype; and the Siberian subtype (Gritsun et al. 1993, Wallner et al. 1995, Gritsun et al. 1997, Ecker et al. 1999, Heinz et al. 2000). In a recent study, other possible genotypes were also identified in Siberia (Tkachev et al. 2011). TBEV is transmitted by tick bites and is maintained in the zoonotic transmission cycle between *Ixodes* ticks and wild vertebrate hosts. Humans are accidental hosts. The most important vertebrate hosts for TBEV are rodents, which have the highest population densities within an endemic area (generally *Apodemus*, *Myodes*, and *Microtus* species).

In 1993, the first confirmed case of serologically diagnosed TBE was reported in Hokuto, Hokkaido Prefecture, Japan (Takashima et al. 1997). TBEV was isolated from dogs, ticks, and rodents in the area where the patient with TBE lived (Takeda et al. 1998, Takeda et al. 1999), and the virus was identified as the Far Eastern subtype of TBEV after nucleotide sequence analysis. Although no TBE case has been reported on Hokkaido since the first case, our epizootiological survey indicated that endemic foci of TBEV were maintained in Hokuto until recently (Yoshii et al. 2011). Therefore, isolating and characterizing TBEV endemic in the area is necessary to evaluate the epidemiological risk of TBE.

In this study, we sought to isolate TBEV from wild rodents captured in southern Hokkaido, and investigated the genetic and biological characteristics of the isolated virus.

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Materials and Methods

Rodent survey

Fifty-nine wild rodents were captured using Sherman box traps in grass and shrub areas within forests bordering on pastures in Hokuto and Setana in 2008 (Fig. 1, Table 1) (Yoshii et al. 2011). The spleens were collected and stored at −80°C until virus isolation.

Virus isolation and identification

The spleens of the wild rodents were homogenized using a cold mortar and pestle and suspended in phosphate-buffered saline (PBS) containing 10% fetal bovine serum (FBS). Homogenates from 3–5 rodents of the same rodent species were pooled and used as the inocula. Each of 10–13 1-day-old suckling mice was inoculated intracerebrally with 20 μL of the inoculum. The mice were observed daily for 14 days, and a moribund mouse was killed and stored at −80°C.

The virus isolate was identified by immunofluorescence assay (IFA) using anti-tick-borne flavivirus antibodies and RT-PCR. Briefly, a 10% suspension of suckling mouse brain was prepared and inoculated onto a monolayer of baby hamster kidney (BHK) cells. After 3 days of incubation, the cells were fixed with 3% paraformaldehyde and permeabilized with 0.2% Triton X-100. After blocking with 2% bovine serum albumin (BSA), the cells were incubated with polyclonal hyperimmune murine ascites fluid from Langat virus-infected mice that is cross-reactive to TBEV, followed by Alexa555-conjugated anti-mouse immunoglobulin G (IgG) antibodies. Viral RNA was extracted from the BHK cells using Isogen (Nippon Gene, Tokyo, Japan) and reverse-transcribed with random primers using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). The TBEV-specific sequence was amplified using Platinum *Taq* polymerase (Invitrogen) using the forward primer, 5′-CGGAGACCTGTCCTTGTTAT-3′ and reverse primer 5′-GTATGCATAATTGTCATACC-3′.

TABLE 1. ISOLATION OF TBEV FROM WILD RODENTS CAPTURED IN HOKKAIDO IN 2008

	Place of survey		
Rodent species	*Hokuto*	*Setana*	*Total*
Apodemus speciosus	13 (3)[a]	—	13 (3)
Apodemus argenteus	10 (3)	7 (2)	17 (5)
Myodes rufocanus	11 (3)	18 (4)	29 (7)

[a]Number of rodents (number of spleen pools used for isolation).

The nucleic acid sequences of the viral genomes were determined by direct sequencing. The cycle sequencing reactions were performed using a BigDye™ Terminator Cycle Sequencing Kit (Life Technologies, Carlsbad, CA), and the sequences were determined with a 3130 Genetic Analyzer (Life Technologies).

Phylogenetic analysis

A phylogenetic analysis was performed using the complete E gene sequences of the TBEV strains, including strains isolated in Hokkaido (the accession numbers are shown in Fig. 3, below). Omsk hemorrhagic fever virus (OHFV) was used as the outgroup. ClustalX version 2.1 was used to generate the multiple alignments (Thompson et al. 1997), MEGA 4 (www.megasoftware.net/mega.html) was used to generate phylogenetic trees by the neighbor-joining method. The reliability

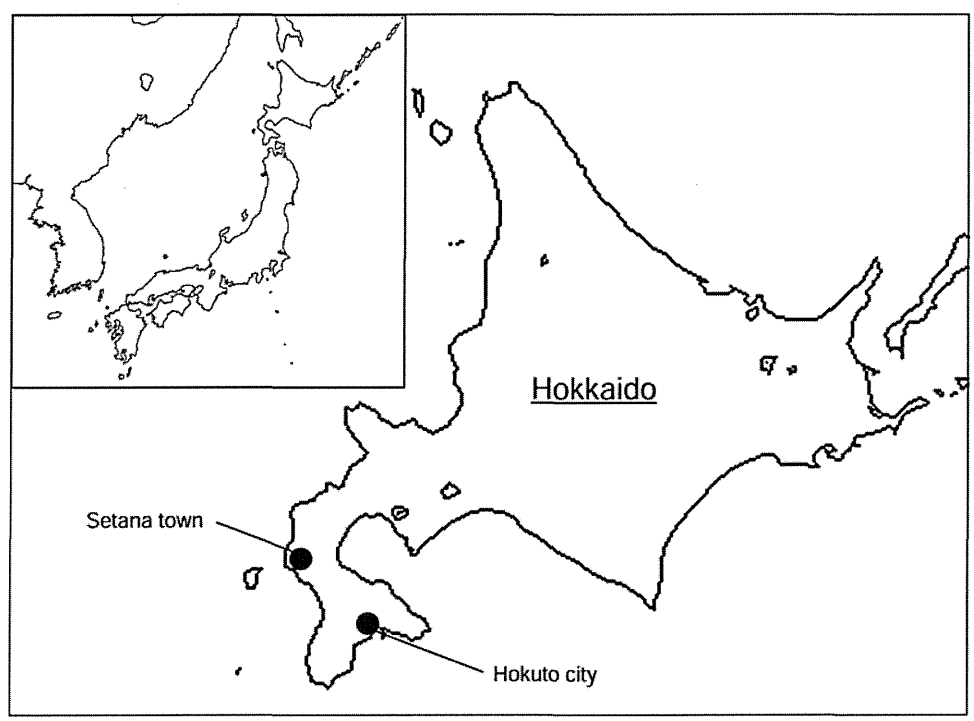

FIG. 1. Geographical location of the study areas.

of the dendrogram was evaluated using 1000 bootstrap replicates.

Growth curve in cell culture

Subconfluent BHK cells were grown in 24-well plates. The cells were inoculated with virus at a multiplicity of infection (MOI) of 0.01 plaque forming unit (pfu). Cells were incubated at 37°C in 5% CO_2. The supernatant was harvested 12, 24, 36, and 48 h postinoculation and stored in aliquots at −80°C before titration.

For titration, cell monolayers prepared in 12-well plates were incubated with serial dilutions of virus for 1 h, and then overlaid with minimal essential medium (MEM) containing 2% FBS and 1.5% carboxymethyl cellulose (CMC; Sigma, St. Louis, MO) and incubated for 5 days. After incubation, the cells were fixed and stained with 0.25% Crystal Violet in 10% buffered formalin. Plaques were counted and expressed as pfu/mL.

Animal model

Viruses were inoculated subcutaneously into 5-week-old female C57BL/6J mice (Jackson ImmunoResearch, West Grove, PA). Morbidity was defined as a >10% weight loss. Surviving mice were monitored for 28 days postinfection to determine survival curves and mortality rates. To analyze the viral distribution in tissues, serum, brains, and spleens were collected from mice 3, 6, 9, and 12 days postinfection. The organs were weighted individually and homogenized, and prepared as 10% suspensions (wt/vol) in PBS that contained 10% FBS. The suspensions were clarified by centrifugation (4000 rpm for 5 min, 4°C), and the supernatants were titrated by plaque assay on BHK cells.

Histopathological examination

Mice infected with 1000 pfu of TBEV were killed 10–11 days postinfection, and fixed brain tissues were embedded in paraffin, sectioned, and stained with Hematoxylin & Eosin, as described previously (Nagata et al. 2007). Immunohistochemical detection of TBEV antigens was performed using rabbit polyclonal antibodies against E protein (Yoshii et al. 2004).

Results

Isolation and identification of TBEV in Hokuto

One suckling mouse became sick after inoculation with 1 group of spleen homogenates of an *Apodemus speciosus* captured in Hokuto. The brain homogenate of this mouse was inoculated into BHK cells to confirm the isolation of TBEV. Viral-specific antigens and a band were detected with an indirect IFA and RT-PCR, respectively (Fig. 2). Consequently, the isolate was identified as TBEV and designated Oshima 08-As.

Genetic analysis of the isolated TBEV

The nucleotide sequence of the complete genome of Oshima 08-As was determined (accession no. AB753012). A phylogenetic tree of the TBEV strains is shown in Fig. 3. Oshima 08-As and the other strains isolated in Hokkaido from 1995 to 1996 (Takashima et al. 1997, Hayasaka et al. 2001) formed a cluster with 100% bootstrap support, and were classified as the Far Eastern subtype of TBEV.

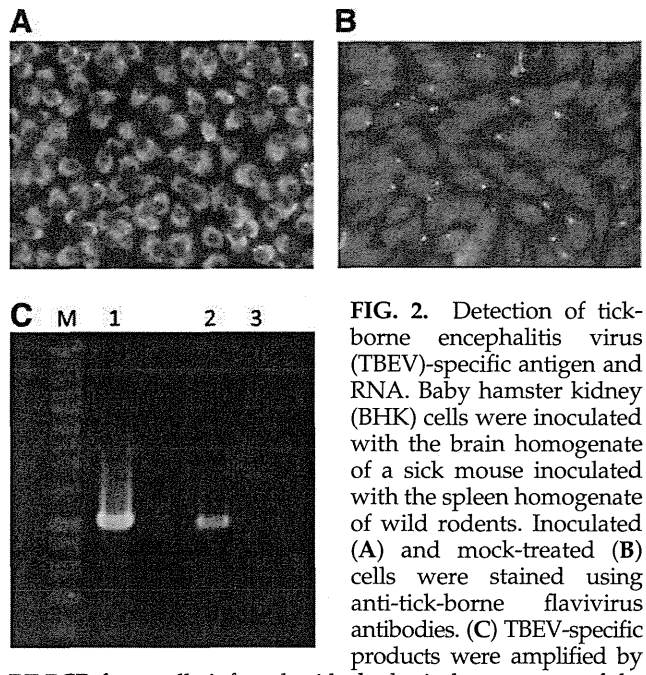

FIG. 2. Detection of tick-borne encephalitis virus (TBEV)-specific antigen and RNA. Baby hamster kidney (BHK) cells were inoculated with the brain homogenate of a sick mouse inoculated with the spleen homogenate of wild rodents. Inoculated (**A**) and mock-treated (**B**) cells were stained using anti-tick-borne flavivirus antibodies. (**C**) TBEV-specific products were amplified by RT-PCR from cells infected with the brain homogenate of the sick mouse (lane 1). TBEV-infected (lane 2) and mock-treated (lane 3) cells were used as positive and negative controls, respectively.

The sequence of Oshima 08-As was compared with that of the Oshima 5–10 strain isolated from a dog in 1995 in Hokuto (Table 2) (Takashima et al. 1997). Thirty-six nucleotide differences resulted in 12 amino acid changes. All of the amino acid changes were located in the region encoding the nonstructural (NS) proteins.

Growth properties of Oshima 08-As

The growth properties of Oshima 08-As were compared with those of Oshima 5–10 by monitoring the virus release after infection. BHK cells were infected with TBEV at a MOI of 0.01. Virus was harvested at 12-h intervals, and the yield was quantified using plaque assay (Fig. 4). The growth curves indicate similar growth properties for Oshima 08-As and Oshima 5–10.

Pathogenicity of Oshima 08-As

The pathogenicity of Oshima 08-As was examined in a mouse model. Mice were infected subcutaneously with serially diluted (10^2–10^6 pfu/mouse) Oshima 08-As or Oshima 5–10 and were monitored for 28 days (Fig. 5, Table 3). All mice infected with each dose of Oshima 08-As showed general signs of illness, such as a hunched posture, ruffled fur, and general malaise. Dose-independent morbidity (60–100%) was observed, and many dying mice infected with Oshima 08-As showed neurological signs, such as paralysis and loss of balance. Several mice died after a sharp decrease in body weight before displaying neurological signs. In contrast, lower mortality and morbidity were observed in the mice infected

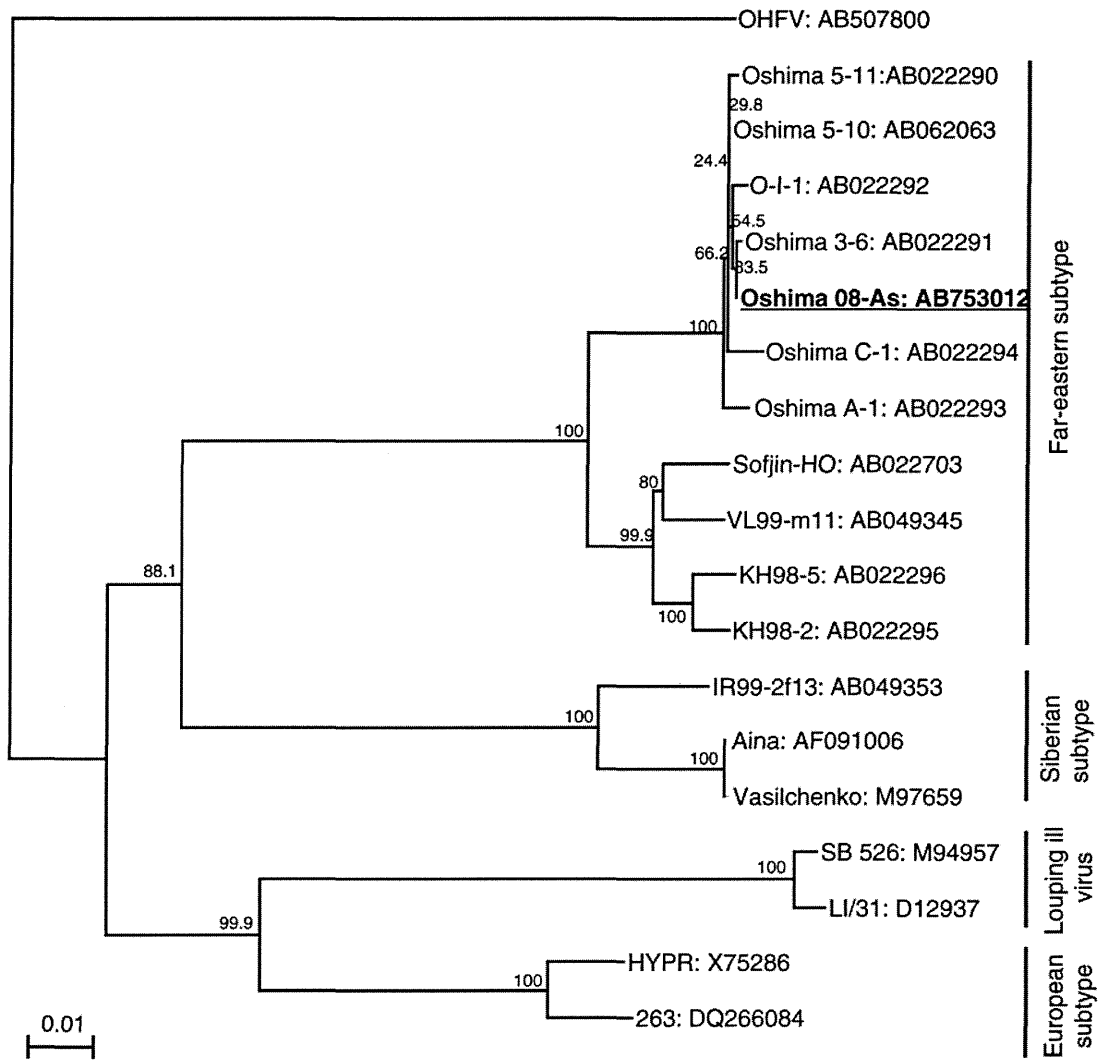

FIG. 3. Phylogenetic tree of the tick-borne encephalitis virus (TBEV) strains The tree was constructed using 1488 nucleotides of the viral *E* gene and Omsk hemorrhagic fever virus (OHFV) as the outgroup. Horizontal distances are proportional to the minimum number of nucleotide differences. The numbers beside the branches are bootstrap values. Accession numbers are shown after the virus strains.

with Oshima 5–10. Several mice recovered after a slight weight loss.

To examine the correlation between disease development and viral replication in organs, the viral loads in the blood, spleen, and brain were compared in mice inoculated with 1000 pfu of Oshima 08-As and Oshima 5–10 (Fig. 6). Viremia was barely observed in both of the mice infected with each virus. Virus was detected in the serum of 1 mouse each infected with Oshima 08-As (4.8×10^4 pfu/mL) or Oshima 5–10 (2.9×10^4 pfu/mL) at 3 days postinfection (dpi). Similar transient multiplication in the spleen was observed in the mice infected with Oshima 08-As and Oshima 5–10 (Fig. 6A). In the brain, the virus was detected from 6 dpi and multiplied until 12 dpi (Fig. 6B). Although statistically significant difference was not observed, the virus titer was higher in the mice infected with Oshima 08-As than in those infected with Oshima 5–10 at each time postinfection.

The histopathological features of the infected mice were examined following infection with 1000 pfu of Oshima 08-As or Oshima 5–10 at 10 dpi. Meninges inflammation and perivascular cuffing were observed in the brains of all mice (4/4) infected with Oshima 08-As (Fig. 7A). Viral antigens were detected in neuronal cells (Fig. 7B), and the virus titer in the brain was 3.8×10^6 pfu/mL. In contrast, minimal inflammatory reaction and few viral antigen-positive cells were observed in the brains infected with Oshima 5–10 (Fig. 7C, D). The viral load in the brain was 4.2×10^5 pfu/mL. These results indicated that Oshima 08-As is more virulent than Oshima 5–10.

Discussion

It has been shown that rodents can be used a useful indicator of the circulation of TBEV in an area (Achazi et al. 2011, Knap et al. 2012). In this study, TBEV was isolated from *A. speciosus* captured in Hokuto in 2008. One virus was isolated from 34 wild rodents (2.9%). This ratio of isolation was similar to that in a survey conducted in the same area in 1995–1996 (1.2%: 2/169) (Takeda et al. 1999). The proportion of rodents

TABLE 2. NUCLEOTIDE DIFFERENCES BETWEEN OSHIMA 08-AS AND OSHIMA 5–10

		Oshima-08-As		*Oshima-5–10*	
Position	*Gene*	*Nucleotide*	*Amino acid*[a]	*Nucleotide*	*Amino acid*
73	5′-UTR	c	—	t	—
431	Core	c	Asp	t	Asp
1730	Env	t	Asp	c	Asp
1847		t	Val	a	Val
2631	NS1	g	**Val**	t	**Leu**
3855	NS2A	t	Leu	c	Leu
4151		c	Gly	t	Gly
4188		a	**Ile**	g	**Val**
4731	NS3	c	**Leu**	t	**Phe**
4968		a	**Arg**	g	**Gly**
4982		a	Ala	g	Ala
5213		a	Lys	g	Lys
5323		a	**Asn**	g	**Ser**
5753		a	Gly	g	Gly
5768		t	Cys	c	Cys
5988		a	**Ile**	g	**Val**
6051		a	**Ser**	g	**Gly**
6524	NS4A	t	Val	c	Val
6583		g	**Arg**	a	**Lys**
7236	NS4B	t	Leu	c	Leu
7385		g	Gly	a	Gly
7587, 7589		g, t	**Gly**	a, c	**Ser**
7616		c	Phe	t	Phe
7898	NS5	t	Val	g	Val
7966		a	**Lys**	g	**Arg**
8462		t	Val	c	Val
8615		g	Ser	a	Ser
9233		g	Leu	a	Leu
10109		c	Asn	t	Asn
10175		c	Val	t	Val
10249		a	**Lys**	g	**Arg**
10300		c	**Thr**	a	**Lys**
10476	3′-UTR	a	—	g	—
10823		a	—	g	—
10843		g	—	a	—

[a]Different amino acids between Oshima 08-As and Oshima 5–10 are shown in bold type.
UTR, untranslated region.

seropositive for TBEV in Hokuto was 12.4% in 1995–1996 and 10.4% in 2008 (Takeda et al. 1999, Yoshii et al. 2011). These data demonstrated that TBEV has been endemic in wild rodents and ticks in Hokuto.

The complete sequence analysis revealed 36 nucleotide differences resulting in 12 amino acid changes between the Oshima 08-As and Oshima 5–10 strains. These differences were consistent with the average synonymous substitution rate estimated previously in the Far Eastern subtype of TBEV (2.9×10^{-4} per site per year) (Hayasaka et al. 1999). All of the amino acid changes were located in the NS proteins. The genetic stability of the viral structural proteins may be important for transmission in wild rodents and ticks.

In the mouse model, Oshima 08-As was more virulent than Oshima 5–10 isolated in 1995. Although the multiplication in the blood and peripheral organs was similar for both strains, the multiplication in the brain was slightly higher in Oshima 08-As than in Oshima 5–10. These data imply that Oshima 08-As was more neuroinvasive than Oshima 5–10 or that Oshima 08-As replicates more efficiently in neural cells. Early inflammatory responses were observed with the viral multiplication in brain infected with Oshima 08-As, compared with the mice infected with Oshima 5–10. In our previous study, systemic inflammatory and stress responses were involved in fatal infection following the subcutaneous infection of mice with the Oshima 5–10 strain (Hayasaka et al. 2009). The induction of the systemic inflammatory and stress responses by viral multiplication might be involved in the greater virulence of Oshima 08-As. It has been shown that naturally occurring mutations affect the pathogenic properties of circulating TBEV (Gritsun et al. 2003, Ruzek et al. 2008, Ruzek et al. 2009). Only 12 amino acid differences exist between Oshima 08-As and Oshima 5–10, but none of them are previously reported amino acid residues that affect the virulence of TBEV. The identification of critical residues that affect the virulence might contribute to the understanding regarding the pathogenicity of the Oshima strains.

Our results indicated that naturally occurring virulent TBEV strains co-circulate among attenuated strains in endemic areas of Japan, as shown in the study by Ruzek et al.

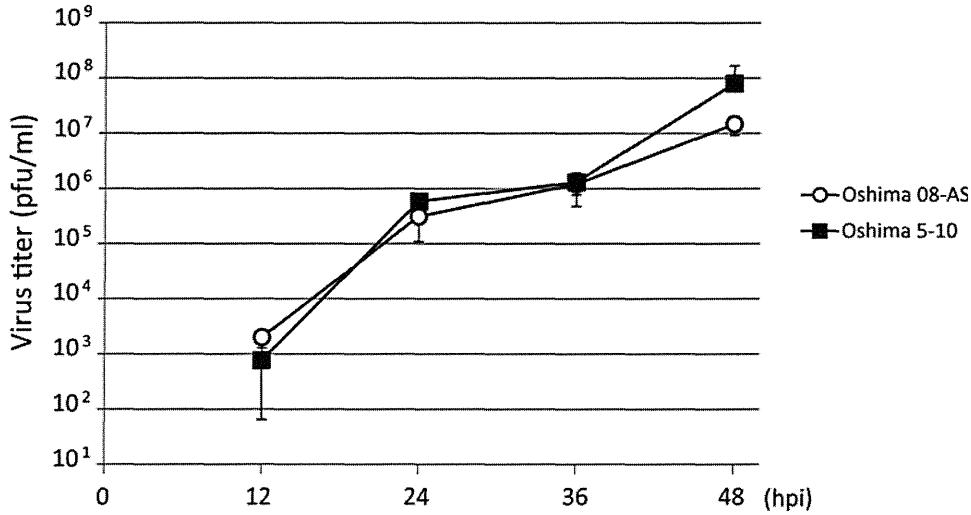

FIG. 4. Comparison of the growth curves of Oshima 08-As and Oshima 5–10. A monolayer of baby hamster kidney (BHK) cells was infected with each virus at a multiplicity of infection (MOI) of 0.01. At each time point, the medium was harvested and virus titers were determined using a plaque assay in BHK cells.

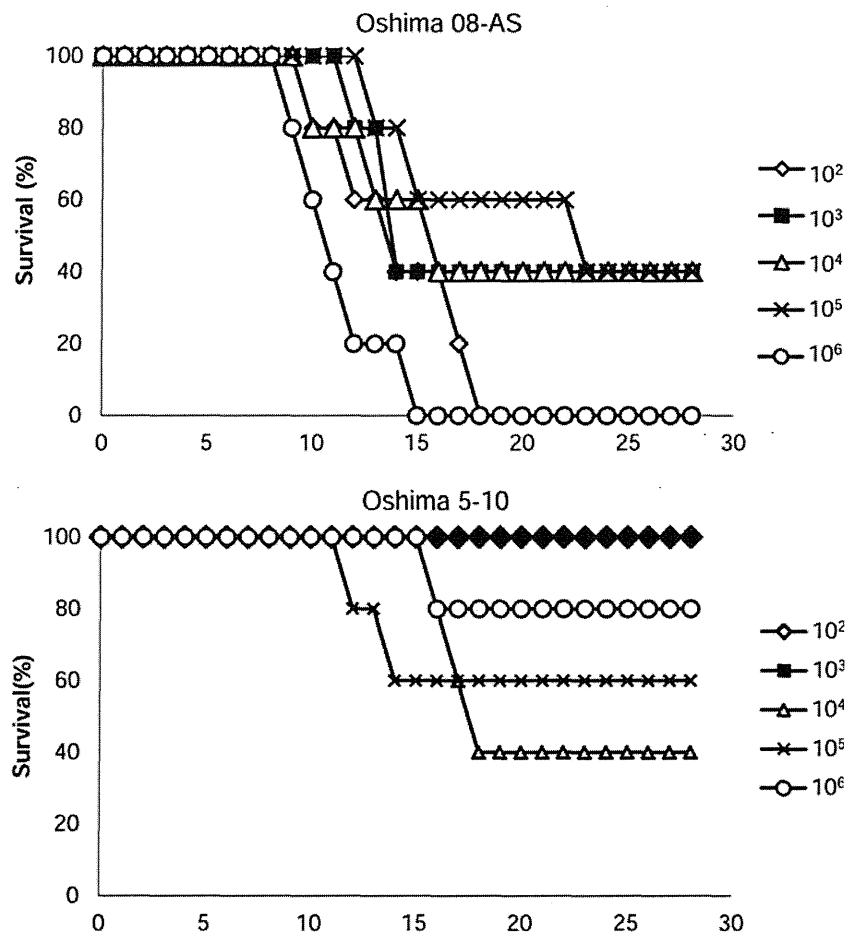

FIG. 5. The survival of mice inoculated with Oshima 08-As and Oshima 5–10. Mice were inoculated subcutaneously with 10^2 to 10^6 plaque-forming units (pfu) of each virus and were monitored for 28 days.

TABLE 3. MORTALITY AND MORBIDITY FOLLOWING SUBCUTANEOUS INFECTION WITH OSHIMA 08-AS AND OSHIMA 5–10 IN B6 MICE

	Oshima 08-AS				*Oshima 5–10*			
Dose (pfu)	*Morbidity*[a] *(%)*	*Mortality (%)*	*Day of onset (days)*	*Survival time (days)*	*Morbidity (%)*	*Mortality (%)*	*Day of onset (days)*	*Survival time (days)*
10^2	100 (5/5)[b]	100 (5/5)[c]	10.0±1.2	13.2±3.4	0 (0/5)	0 (0/5)	—	—
10^3	100 (5/5)	60 (3/5)	10.2±1.6	12.3±1.2	40 (2/5)	0 (0/5)	14.5±0.7	—
10^4	100 (5/5)	60 (3/5)	9.0±0.7	12.0±3.0	100 (5/5)	60 (3/5)	11.8±1.3	16.0±1.0
10^5	100 (5/5)	60 (3/5)	8.8±0.8	16.0±5.3	60 (3/5)	40 (2/5)	10.8±2.9	12.0±1.4
10^6	100 (5/5)	100 (5/5)	7.6±0.6	10.4±2.3	80 (4/5)	20 (1/5)	11.5±1.7	15

[a]Morbidity of mice was estimated by >10% of weight loss.
[b]Number of sick mice/number of infected mice.
[c]Number of dead mice/number of infected mice.

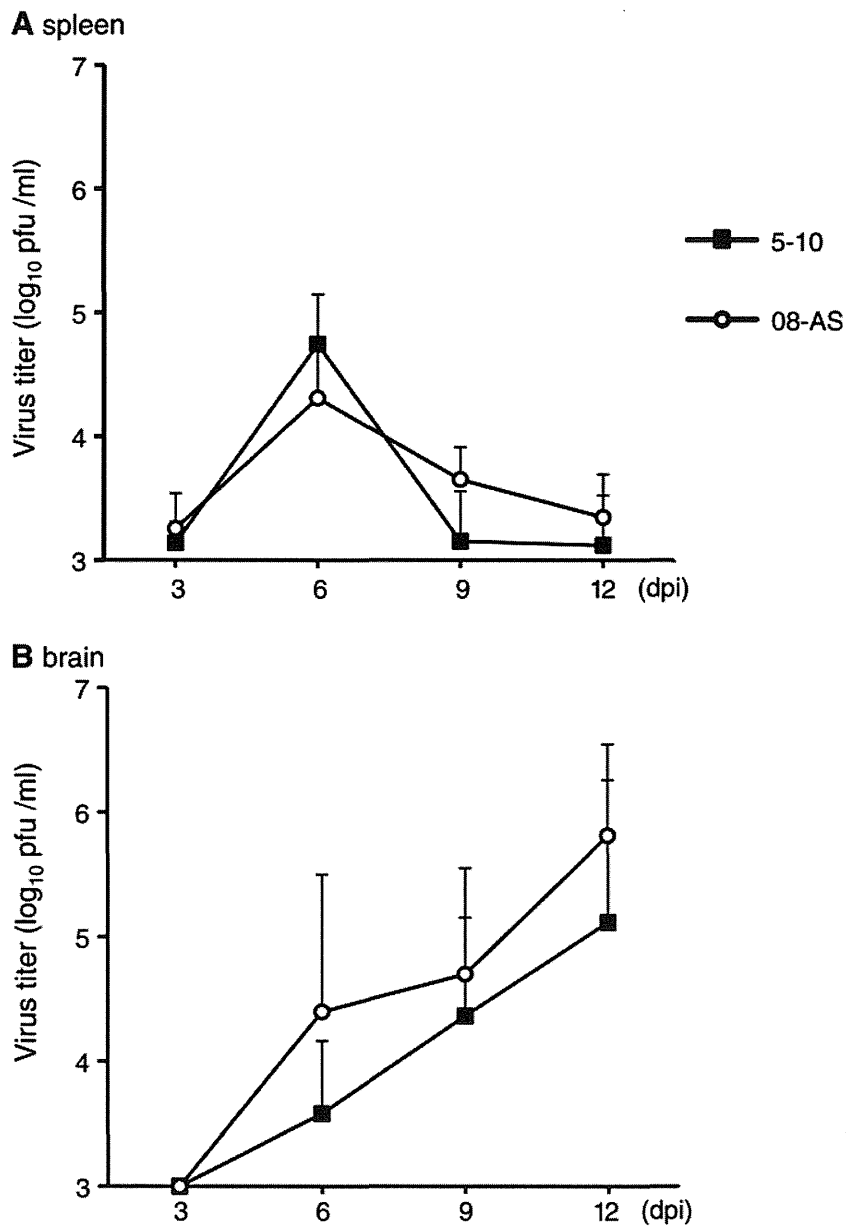

FIG. 6. Virus replication in organs. Mice were infected with 1000 pfu of Oshima 08-As or Oshima 5–10. Virus titers in spleen (**A**) and brain (**B**) at the indicated days after infection were determined using plaque assays. Error bars represent the standard deviation (SD) ($n=3$).

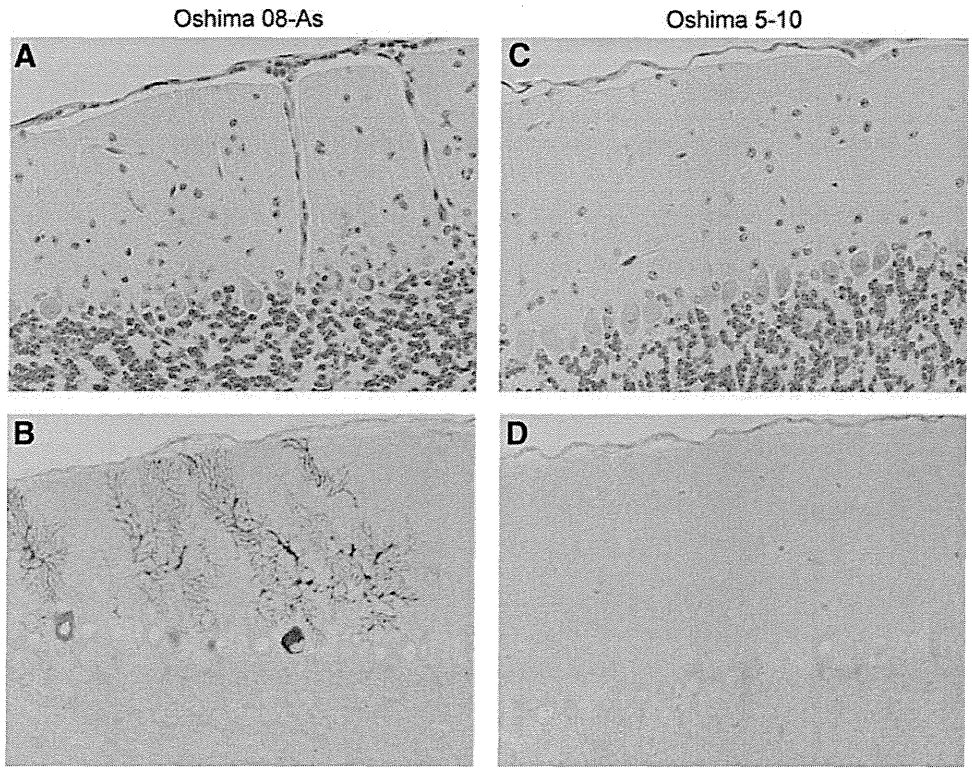

FIG. 7. Histopathological features in the brain at 10 days post-infection. B6 mice were infected with 103 pfu of the Oshima 08-As (**A** and **B**) or Oshima 5–10 (**C** and **D**) strain. TBEV antigens were detected using E protein-specific antibodies (lower columns: **B** and **D**).

(2008). Although no human TBE cases have been reported in Japan since 1993, our study clearly demonstrated that TBEV is silently circulating in Hokkaido. It is possible that inhabitants in the endemic area may have been infected by attenuated TBEV strains and acquired immunity against TBEV.

In summary, we isolated TBEV strain Oshima 08-As from a wild rodent captured in Hokuto in 2008 and showed that a few naturally occurring mutations affect the virulence of the endemic Oshima strains. These results are important for monitoring TBEV to evaluate the epidemiological risk in the endemic area of Hokkaido.

Acknowledgment

This work was supported by Grants-in-Aid for Scientific Research (22780268 and 21405035) and the Global COE Program from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan, and Health Sciences Grants for Research on Emerging and Re-emerging Infectious Disease from the Ministry of Health, Labour and Welfare of Japan.

Author Disclosure Statement

No competing financial interests exist for this paper.

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Arch Virol (2013) 158:1039–1046
DOI 10.1007/s00705-012-1579-1

ORIGINAL ARTICLE

Susceptibility to flavivirus-specific antiviral response of Oas1b affects the neurovirulence of the Far-Eastern subtype of tick-borne encephalitis virus

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Received: 12 June 2012 / Accepted: 10 October 2012 / Published online: 25 December 2012

Abstract Tick-borne encephalitis virus (TBEV) is a zoonotic agent that causes fatal encephalitis in humans. 2'-5'-oligoadenylate synthetase 1b (*Oas1b*) has been identified as a flavivirus resistance gene, but most inbred laboratory mice do not possess a functional *Oas1b* gene. In this study, a congenic strain carrying a functional *Oas1b* gene, B6.MSM-*Oas*, was used to evaluate the pathogenicity of Far-Eastern TBEV. Although intracerebral infection of B6.MSM-*Oas* mice by Oshima 5-10 resulted in limited signs of illness, infection by Sofjin-HO resulted in death with severe neurologic signs. While Oshima 5-10 was cleared from the brain, Sofjin-HO was not cleared despite a similar level of expression of the intact *Oas1b* gene. Necrotic neurons with viral antigens and inflammatory reactions were observed in the brain infected with Sofjin-HO. These data indicate that the different susceptibility to the antiviral activity of Oas1b resulted in a difference in neurovirulence in the two TBEV strains.

Abbreviations

BHK	Baby hamster kidney
B6	C57BL/6J
CNS	Central nervous system
FCS	Fetal calf serum
LD_{50}	50 % lethal dose
MEM	Minimum essential medium
OAS	2'-5'-oligoadenylate synthetase
Oas1b	2'-5'-oligoadenylate synthetase 1b
pfu	Plaque-forming unit
TBE	Tick-borne encephalitis
TBEV	Tick-borne encephalitis virus
TBST	TBS containing 0.01% Tween 20
WNV	West Nile virus

Introduction

Tick-borne encephalitis virus (TBEV) is a member of the genus *Flavivirus* within the family *Flaviviridae*. Tick-borne encephalitis (TBE) is endemic in Europe, Russia, and Far-East Asia, including Japan, and about 10,000 cases of the disease are reported every year on the Eurasian continent [28]. TBEV has been subdivided into three subtypes: the Far-Eastern subtype, which causes Russian spring-summer encephalitis in Russia; the western European subtype; and the Siberian subtype [5, 9]. Infection with the Far-Eastern subtype of the virus causes severe encephalitis; case fatality rates are reported to be 20–60 %. Thus, TBE is a significant public-health problem in these endemic regions.

Electronic supplementary material The online version of this article (doi:10.1007/s00705-012-1579-1) contains supplementary material, which is available to authorized users.

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Our previous studies showed that the Far-Eastern subtype of the TBEV Oshima strain, which was isolated in Japan [30], caused different disease of the central nervous system (CNS) when compared with the prototype strain Sofjin-HO [3, 10]. In addition to the development of CNS disease, some host responses (which were not observed after infection of Sofjin-HO) were shown to be involved in the induction of a fatal infection. However, the detailed mechanisms remain largely unknown. Since the amino acid sequence identity between the two strains is more than 98 % [7], comparative analysis can reveal important information regarding the pathogenicity of TBEV.

Interferon-inducible 2'-5'-oligoadenylate synthetases (OASs) play important roles in the antiviral activity against RNA virus infections. After activation by double-stranded RNA, OAS proteins polymerize adenosine 5'-triphosphate (ATP) into 2'-5'-linked oligoadenylates (2-5As) [22, 24]. These 2-5As activate RNase L, resulting in the degradation of viral RNA [23]. The OAS family consists of *OAS1*, *OAS2*, *OAS3*, and multiple *OAS*-like genes in humans [2, 8, 11], and eight small *Oas1* (*Oas1a-h*), one *Oas2*, one *Oas3*, and two *Oas*-like (*OasL1* and *OasL2*) genes in mice [12].

The murine isoform *Oas1b* has been identified as a critical determinant of the genetic susceptibility of mice to infection with West Nile virus (WNV), a mosquito-borne flavivirus [16, 20]. It was recently reported that genetic variation in human OAS is associated with a predisposition to TBEV- and WNV-induced diseases [1, 15]. However, little is known about the detailed mechanism of OASs in the pathogenesis of tick-borne flaviviruses. Since most inbred laboratory mice do not possess a functional *Oas1b* gene due to a premature stop codon, the susceptibility to flaviviruses is increased. The increased susceptibility has made it difficult to analyze the roles of OASs in flavivirus pathogenesis in detail.

We previously established a congenic strain in which the *Oas* locus of the Japanese feral-mouse-derived strain MSM/Ms was introduced to the widely used mouse strain C57BL/6J (B6) [18]. These B6.MSM-*Oas* mice have a functional *Oas1b* gene and show resistance to infection by WNV but not influenza virus. In this study, B6.MSM-*Oas* mice were used to evaluate the pathogenesis of the Far-Eastern subtype of TBEV, and differences in susceptibility of different strains to the antiviral responses of Oas1b were observed.

Materials and methods

Viruses

The Sofjin-HO strain of TBEV (accession no. 062064) was first isolated from the brain of a TBE patient in Khabarovsk in 1937 [4]. The virus (of unknown passage history) was generously supplied by Dr. Ohya (National Institute of Infectious Diseases, Tokyo, Japan) in 1967; the virus was further passaged seven times in suckling mouse brain and twice in baby hamster kidney (BHK) cells. The Oshima 5-10 strain was isolated from dogs in 1995 in Hokuto City, Japan [30] and was passaged twice in suckling mouse brain and once in BHK cells. Viruses were handled in biosafety level 3 containment. BHK cells were grown at 37 °C in Eagle's minimum essential medium (MEM) supplemented with 8 % fetal calf serum (FCS) and L-glutamine.

Virus infection in mice

Five-week-old female C57BL/6J (B6) (Charles River Laboratories Japan, Inc., Yokohama, Japan) or C57BL/6J.MSM-*Oas* (B6.MSM-*Oas*) mice available from Riken BRC (B6.MSM-[D5Mit367-D5Mit242]/Hkv: RBRC. no. RBRC05266) [18] were anesthetized and then inoculated intracerebrally with a range of 10^1–10^5 plaque-forming units (pfu) of TBEV. The mice were weighed daily and checked for clinical signs for 21 days. Morbidity was defined as the appearance of >10 % weight loss. For analysis of the viral titer and gene expression, three mice were sacrificed on days 3, 6, and 9 post-infection, and brain samples were collected following perfusion with cold PBS and stored at −80 °C. All procedures were approved by the President of Hokkaido University after review by the Animal Care and Use Committee of Hokkaido University.

Viral titration

Brain samples were weighed, homogenized, and prepared as 10 % suspensions (w/v) in PBS supplemented with 10 % FCS. The suspensions were clarified by centrifugation (4,000 rpm for 5 min at 4 °C), and the viruses in the supernatants were titrated.

Plaque assays were performed with BHK cells using 12-well plates. Serial tenfold dilutions of the organ suspensions were inoculated to the monolayer of cells. After incubation for 1 h at 37 °C, 1.5 % carboxymethylcellulose-MEM was added to the cells. The incubation was continued for four days, and the monolayers were stained with 0.1 % crystal violet in 10 % formalin neutral buffer solution. Plaques were counted, and infectivity titers were expressed as pfu/mL.

Semi-quantitative RT-PCR

Total brain tissue RNA isolated by Isogen (Nippon Gene, Tokyo, Japan) was used for RT-PCR. Equal amounts (0.2 μg) of RNA were subjected to reverse transcription using SuperScript II and Oligo(dT)$_{20}$ Primer (Life

Technologies, Carlsbad, CA, USA) at 42 °C for 50 min and 70 °C for 15 min, followed by 26 (β-actin) and 35 (*Oas1a* and *Oas1b*) PCR cycles consisting of 94 °C for 30 s, 55 °C for 30 s and 68 °C for 2 min using Platinum *Taq* (Life Technologies). The following primers were used: β-actin-forward, 5'-CATGAACAACAGGTGGATCCTCC ACGC-3'; β-actin-reverse, 5'-CAGTTTTGGAAGTTTCT GGTAAGTCTTCG-3'; *Oas1a*-forward, 5'-TGTTAATAC TTCCAGCAAGC-3'; *Oas1a*-reverse, 5'-GCAAAGACAG TGAGCAACTCT-3'; *Oas1b*-forward, 5'-AGGCTGCCGC CTGGCTGCAAT-3'; *Oas1b*-reverse, 5'-TAAGGCAGG AGGATGGCAATA-3'.

Histopathological examination

Mice infected with 10^4 pfu of TBEV were killed at 9 days post-infection, and fixed brain tissues were embedded in paraffin, sectioned and stained with haematoxylin and eosin as described previously [19]. Immunohistochemical detection of TBEV antigens was performed using rabbit polyclonal antibodies against E protein to detect TBEV antigens [31].

Results

Differential resistance of *Oas*-congenic mice to the neurovirulence of the TBEV strains

Initially, B6.MSM-*Oas* mice were subcutaneously infected with the Sofjin-HO or Oshima 5-10 strain of TBEV. Following infection with 10^6 pfu of either strain, all mice survived without any clinical signs. No viremia or viral multiplication in organs (spleen, lung, liver, and brain) was observed (data not shown). These data indicate that, in B6.MSM-*Oas* mice, the virus was eliminated in the early stage of infection following subcutaneous challenge.

To evaluate the neurovirulence of TBEV, B6 and B6.MSM-*Oas* mice were intracerebrally infected with serial doses of the Sofjin-HO or Oshima 5-10 strain. Although some B6 mice survived at low doses of infection (10 and 100 pfu), most of the mice died following intracerebral infection with either TBEV strain (Table 1). All mice showing signs of illness died within 2 days from the onset of the disease. The mice that survived at a low dose of infection did not show any clinical signs of disease. The 50 % lethal dose (LD_{50}) in B6 mice was 16.2 pfu for Sofjin-HO and 20.9 pfu for Oshima 5-10. In the B6.MSM-*Oas* mice infected with the Sofjin-HO strain, some mice showed resistance to viral infection at a low dose (LD_{50} = 148 pfu). All mice at 10 pfu of infection and 60 % of the mice at 100 pfu of infection survived without any signs of illness; however, 40 % of the mice at 100 pfu of infection and all mice at more than 1,000 pfu of infection died. In contrast, most of the B6.MSM-*Oas* mice infected with 1,000 pfu or more of the Oshima 5-10 strain showed general signs of illness, but all of the mice survived.

Figure 1 shows survival curves (a) and weight changes (b) following intracerebral infection with 10^4 pfu of TBEV. B6 mice infected with both virus strains and B6.MSM-*Oas* mice infected with the Sofjin-HO strain remained asymptomatic for 4–6 days and then started to exhibit general signs of illness, including weight loss, a hunched posture, ruffled fur, and general malaise. The mice lost weight rapidly and died within 3–4 days from onset of disease. Most of the mice (>80 % for each group) exhibited neurological signs of paralysis before death. In contrast, the B6.MSM-*Oas* mice infected with Oshima 5-10 remained asymptomatic for 5–6 days and then started to exhibit general signs of illness, including weight loss, a hunched posture, ruffled fur, and general malaise. However, the weight decrease was very slight compared to the other groups, and all of the mice recovered at 11–14 days post-infection. No neurological signs were observed. These data indicated that in the B6.MSM-*Oas* mice, neurovirulence was different for the two strains of TBEV.

Viral clearance from *Oas*-congenic mouse brain

To examine reduction of viral replication caused by the expression of a functional *Oas1b* gene, viral loads in the brain were investigated after intracerebral infection with 10^4 pfu of TBEV. At 3 days after infection, there were no significant differences in the viral loads between the B6 and B6.MSM-*Oas* mice and between the Sofjin-HO and Oshima 5-10 strains (Fig. 2). At 6 days post-infection, a lower titer of virus was observed in the B6.MSM-*Oas* mice infected with the Oshima 5-10 strain than in the B6 mice, while similarly high levels of virus (>10^7 pfu/mL) were detected in both the B6 and B6.MSM-*Oas* mice infected with Sofjin-HO. This trend was more apparent at 9 days post-infection. The viral loads in the B6.MSM-*Oas* mice infected with the Oshima 5-10 strain were drastically reduced, and the virus was cleared from the brain in two of the four mice. However, the viral loads in the B6.MSM-*Oas* mice infected with the Sofjin-HO strain remained as high as in the B6 mice.

To examine whether the difference in viral clearance in the B6.MSM-*Oas* mice between the Sofjin-HO and Oshima 5-10 strains was due to the expression level of *Oas1b*, the expression of *Oas1a* and *Oas1b* was analyzed by semi-quantitative RT-PCR. As shown in Fig. 3, the expression levels of both *Oas1a* and *Oas1b* increased from 3 to 6 days post-infection, and there was no difference between infection with Sofjin-HO and Oshima 5-10. Oas1a protein

Table 1 Mortality and morbidity following intracerebral infection with the Sofjin-HO and Oshima 5-10 strains of TBEV in B6 and B6. MSM-*Oas* mice

Dose (pfu)	B6 MSM-Oas								B6							
	Sofjin-HO				Oshima 5-10				Sofjin-HO				Oshima 5-10			
	Morbidity[a]		Mortality[b]		Morbidity		Mortality		Morbidity		Mortality		Morbidity		Mortality	
10	0/5	(0 %)	0/5	(0 %)	0/5	(0 %)	0/5	(0 %)	3/5	(60 %)	3/5	(60 %)	2/5	(40 %)	2/5	(40 %)
100	2/5	(40 %)	2/5	(40 %)	0/5	(0 %)	0/5	(0 %)	3/5	(60 %)	3/5	(60 %)	4/5	(80 %)	4/5	(80 %)
1,000	5/5	(100 %)	5/5	(100 %)	5/6	(83.3 %)	0/6	(0 %)	5/5	(100 %)	5/5	(100 %)	5/5	(100 %)	5/5	(100 %)
10,000	11/11	(100 %)	11/11	(100 %)	10/10	(100 %)	0/10	(0 %)	5/5	(100 %)	5/5	(100 %)	5/5	(100 %)	5/5	(100 %)
100,000	5/5	(100 %)	5/5	(100 %)	6/6	(100 %)	0/6	(0 %)	5/5	(100 %)	5/5	(100 %)	5/5	(100 %)	5/5	(100 %)
LD_{50} (pfu)	148				–				16.2				20.9			

[a] Morbidity of mice was estimated by >10 % weight loss. No. of sick mice/no. of infected mice

[b] No. of dead mice/no. of infected mice

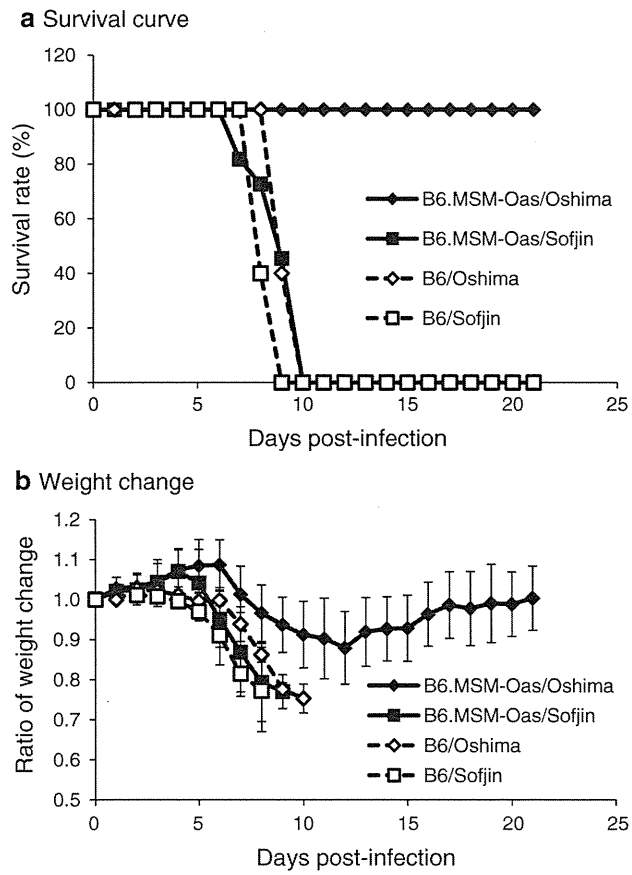

Fig. 1 Survival curves (A) and weight changes (B) following intracerebral infection with TBEV. B6 (open symbol) and B6.MSM-*Oas* (closed symbol) mice were infected with 10^4 pfu of the Sofjin-HO (diamond) or Oshima 5-10 (square) strain and monitored for 21 days. The average daily weight changes are represented as a ratio to the weight at day 0. Error bars represent the standard deviations

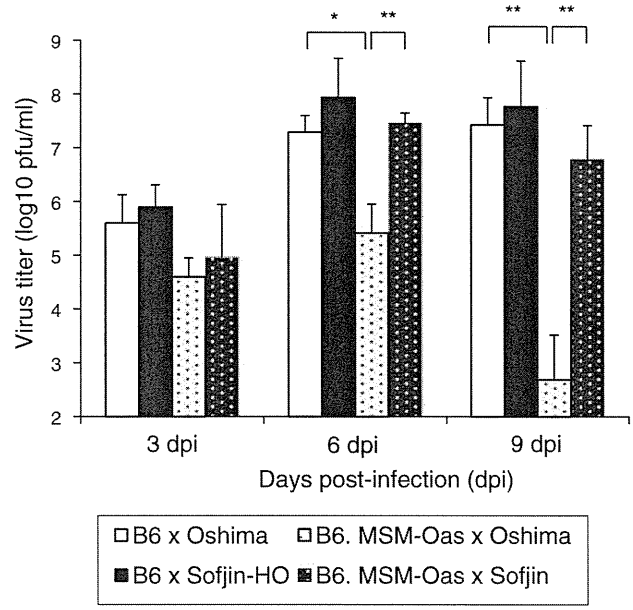

Fig. 2 Virus replication in the brain after intracerebral infection. B6 or B6.MSM-*Oas* mice were infected with 10^4 pfu of the Sofjin-HO or Oshima 5-10 strain. Virus titers in the brain at the indicated days after infection were determined by plaque assays. Error bars represent the standard deviations (n = 4). The symbols * and ** indicate p-values of <0.05 and <0.01, respectively, by the Tukey test and Scheffé F-test

expression was also similar after infection of Sofjin-HO or Oshima 5-10 (Supplementary Fig. 1). These data indicated that both the Sofjin-HO and Oshima 5-10 strains possess a comparable ability to induce *Oas1b* expression. However, the Oshima 5-10 strain was cleared from the brain as intact *Oas1b* was expressed, whereas the Sofjin-HO strain was not cleared despite a similar level of expression of intact *Oas1b*.

Histopathological features of the B6.MSM-*Oas* mice

The histopathological features of the B6.MSM-*Oas* mice were examined following intracerebral infection with the

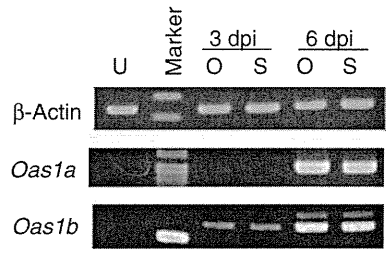

Fig. 3 Expression of *Oas* genes in the brain following intracerebral infection. B6.MSM-*Oas* mice were infected with 10^4 pfu of the Sofjin-HO or Oshima 5-10 strain. Total brain tissue RNA (0.2 μg/reaction) from uninfected mice (U) and mice infected with the Sofjin-HO (S) or Oshima 5-10 (O) strain at 3 and 6 days post-infection (dpi) were subjected to semi-quantitative RT-PCR for β-actin, *Oas1a*, and *Oas1b*

Sofjin-HO or Oshima 5-10 strain at 9 days post-infection. In the B6.MSM-*Oas* mice infected with Oshima 5-10, viral-antigen-positive cells were rarely observed throughout the brain (Table 2 and Fig. 4). Perivascular cuffing was observed prominently in the brain as an anti-viral response (Fig. 4a–c), and a number of degenerated Purkinje cells and meningitis were observed in the cerebellum (Fig. 4b). In contrast, many viral-antigen-positive cells and inflammatory reactions were observed throughout the brain of mice infected with the Sofjin-HO strain (Table 2 and Fig. 4d–f). Furthermore, necrotic or degenerated neurons with inflammatory cell infiltration were observed in the cerebrum and cerebellum (Fig. 4d and e).

Compared to the B6 mice infected with Oshima 5-10 or Sofjin-HO (Fig. 5 and Table 2), inflammatory infiltrations were slight in B6.MSM-*Oas* mice infected with Sofjin-HO, but the histopathological signs of neural degeneration and inflammation in Sofjin-HO were similar to those observed in B6 mice infected with either strain. These data suggest that B6.MSM-*Oas* mice infected with the Sofjin-HO strain died due to acute neurological dysfunction throughout the brain; in contrast, the mice infected with the Oshima 5-10 strain survived because the level of viral replication was reduced by the anti-viral activity of *Oas1b*.

Discussion

In this study, we demonstrated that B6.MSM-*Oas* mice, which possess a functional *Oas1b* gene, showed different responses to two different strains of TBEV. In B6.MSM-*Oas* mice infected intracerebrally with the Oshima 5-10 strain, the virus was cleared and *Oas1b* was expressed. All infected B6.MSM-*Oas* mice survived, while most of the virus-infected B6 mice died. Dose-dependent morbidity was observed during the viral clearance phase, while the clinical manifestation was transient with general signs of disease, including weight loss, without any neurological signs; these general signs were considered to be associated with the host's anti-viral response, including inflammation, as observed by histopathological analysis. These results are consistent with those of previous studies of flavivirus-resistant mice infected intracerebrally with mosquito-borne flaviviruses [26, 27].

In contrast, the B6.MSM-*Oas* mice showed no resistance to infection with the Sofjin-HO strain. The infected mice died in a dose-dependent manner, and the fatality rate was 100%. Many mice showed neurological signs, including paralysis, and severe cytopathic effects were observed in the virus-infected neurons with inflammatory responses throughout the brain, as observed in B6 mice. Unlike infection with Oshima 5-10, the viral titer of Sofjin-HO in the B6.MSM-*Oas* mice was not reduced, similar to the effect in B6 mice, despite expression of the functional *Oas1b* gene. These data suggest that the anti-flavivirus activity of *Oas1b* successfully reduced the level of viral replication of the TBEV Oshima 5-10 strain, whereas the Sofjin-HO strain overcame and/or escaped the reduction of

Table 2 Localization of viral antigen and inflammatory reactions in the CNS of the mice following intracerebral infection with 10^4 pfu of TBEV at 9 days post-infection

Mouse	TBEV strain	Days p.i.	Number of positive animals with virus antigen (Number of animals with inflammatory reactions), n = 4						
			Thalamus	Hippocampus	Cerebrum	Mesencephalon	Cerebellum	Pons	Medulla
B6. MSM-*Oas*	Oshima 5-10	9	4	0	1	0	2	0	0
			(4)	(4)	(4)	(4)	(4)	(3)	(3)
	Sofjin-HO	9	4	3	4	2	4	3	2
			(4)	(4)	(4)	(4)	(4)	(4)	(3)
B6	Oshima 5-10	9	4	4	4	4	4	4	4
			(4)	(4)	(4)	(4)	(4)	(4)	(4)
	Sofjin-HO	9	4	4	4	4	4	4	4
			(4)	(4)	(4)	(4)	(4)	(4)	(4)

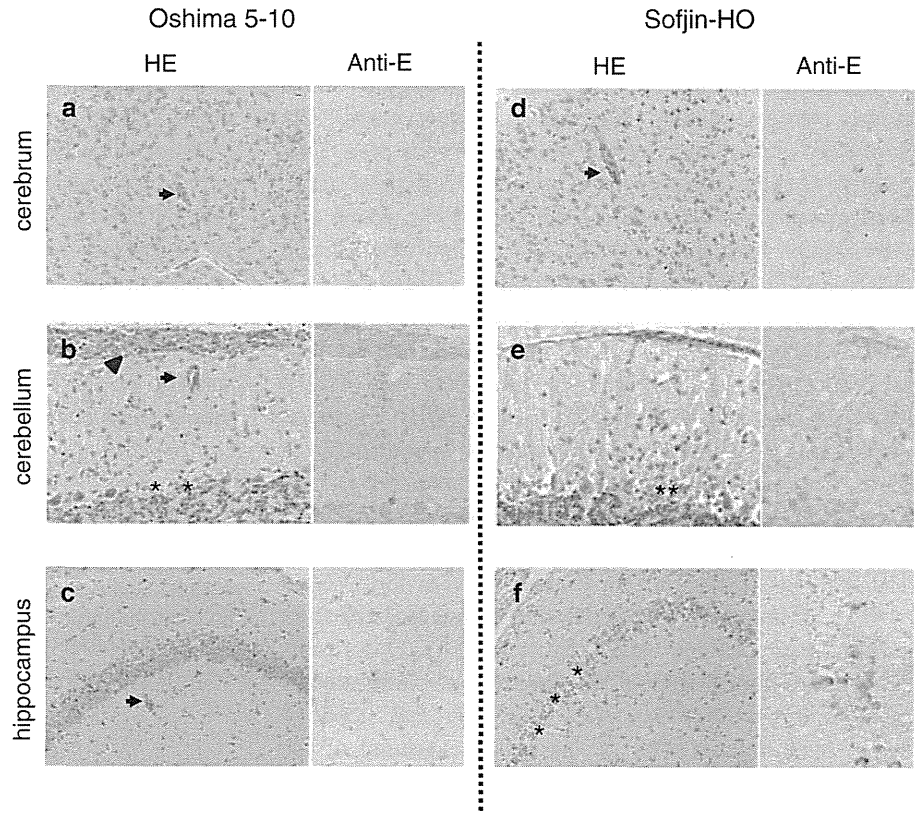

Fig. 4 Histopathological features in the brain of B6.MSM-*Oas* mice following intracerebral infection at 9 days post-infection. B6.MSM-*Oas* mice were infected with 10^4 pfu of the Oshima 5-10 (A–C) or Sofjin-HO (D–F) strain. TBEV antigens were detected using E-protein-specific antibodies (right columns). Meningitis in the cerebellum (B, arrowhead) and perivascular cuffing (arrows) were observed in the mice infected with Oshima 5-10. Necrotic or degenerated neurons (asterisks) with infiltration by inflammatory cells were observed in the mice infected with Sofjin-HO

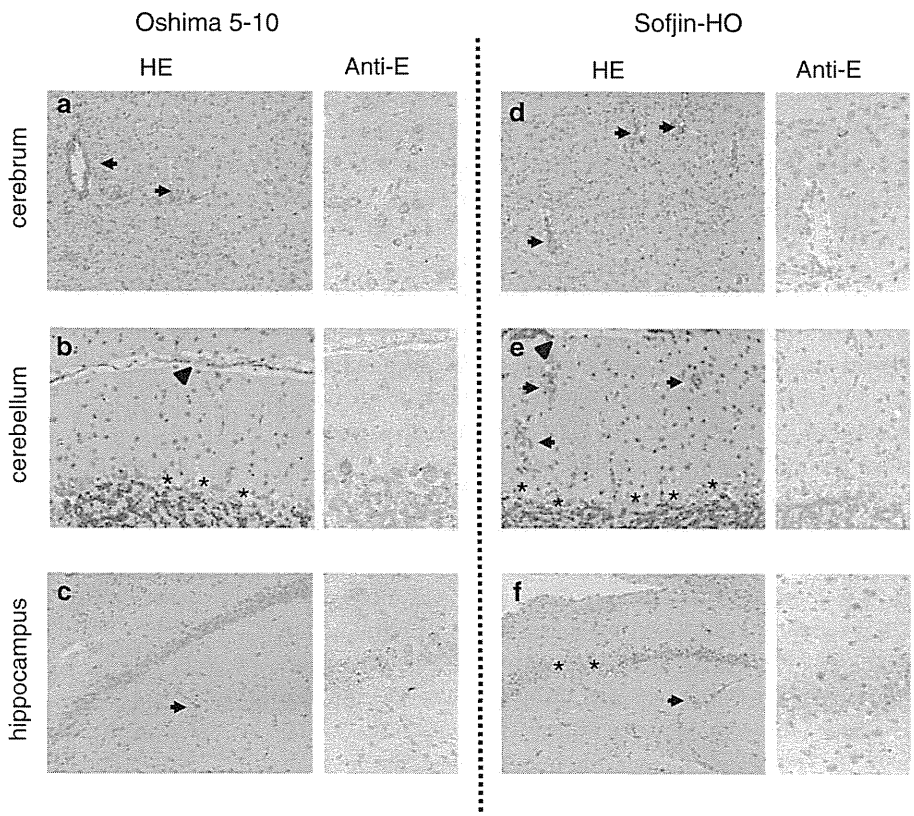

Fig. 5 Histopathological features in the brain of C57BL/6 mice following intracerebral infection at 9 days post-infection. C57BL/6 mice were infected with 10^4 pfu of the Oshima 5-10 (a–c) or Sofjin-HO (d–f) strain. TBEV antigens were detected using E-protein-specific antibodies (right columns. Slight or severe meningitis (b and e, arrowhead) and inflammatory infiltrations (a, c, d, e, and f, arrows) were observed in the mice infected with either strain. Degenerated cells, necrotic neurons, or neuronophagia (b, e, and f, asterisks) were observed mice infected with either strain

viral replication, causing neurological disease in the B6.MSM-*Oas* mice.

The pathogenicity of TBEV in B6.MSM-*Oas* mice closely resembled that observed in human cases. The Sofjin-HO strain was derived from the brain of a TBE patient [4] and is closely related to another reference strain, Khabarovsk-Obor-4 (accession no. FJ214111), which was also isolated from the brain of a TBE patient with a lethal outcome [13]. The Oshima 5-10 strain was isolated from a sentinel dog in Hokkaido, Japan [30]. Endemic foci of Oshima-related strains have been maintained for more than 10 years in the area [32], but only one human case of TBE has been reported. Therefore, Japanese TBEV isolates, including the Oshima 5-10 strain, have been considered to be relatively avirulent. A recent phylogenic analysis of the Far-Eastern subtype of TBEV revealed that the Sofjin-HO strain is genetically closely related to strains isolated from TBE patients in Far-Eastern Russia, whereas the Oshima 5-10 strain is more closely related to strains isolated from asymptomatic patients bitten by ticks [14]. Our results concerning the pathological features of intracerebral infection in B6.MSM-*Oas* mice, which were not identified in B6 mice, agreed with the genetic background of the strains. Peripheral infection in B6.MSM-*Oas* mice resulted in viral clearance during the initial stage of infection, and the mice showed no signs of disease. This is consistent with previous data for WNV [18] and may be related to the low incidence rate in flavivirus-infected individuals. Our previous data showed that peripheral viral multiplication did not differ between the Sofjin-HO and Oshima 5-10 strains in B6 mice and did not correlate with the progression of disease in TBEV infection [10, 29]. Considering the results of intracerebral infection in B6.MSM-*Oas* mice, it may be possible to distinguish clinical conditions based on the TBEV strain after viral invasion of the brain.

Oas1b is induced by a STAT2-dependent pathway, and does not possess 2-5A synthetic activity [6, 21]. The flavivirus-specific antiviral action of Oas1b is independent of the 2-5A/RNase L pathway, which is important for broad nonspecific antiviral activity [25]. An alternative mechanism for this flavivirus-specific antiviral action has been suggested, but it is not well understood. The viral factor that is the target of the flavivirus-specific antiviral activity of Oas1b is also unknown. Only one study has reported that mutations in the NS3 helicase (NS3-365) and 2K peptide (2K-9) of WNV promoted resistance to the antiviral action of Oas1b [17], but no differences exist in these two amino acids between Sofjin-HO and Oshima 5-10. The protein sequence homology between Sofjin-HO and Oshima 5-10 is >98 %, and this difference likely determines the level of susceptibility to the antiviral action of Oas1b. Therefore, the identification of the determinant underlying the differential pathogenicity observed in B6.MSM-*Oas* mice will help reveal the molecular mechanism of the flavivirus-specific antiviral activity of Oas1b.

In summary, using congenic mice possessing a functional *Oas1b* gene, we demonstrated that intracerebral infection with TBEV caused clinical conditions associated with human infection and that reduction of viral replication by the flavivirus-specific antiviral activity of Oas1b was different for different TBEV strains. This congenic mouse strain may be a useful model for studying the detailed pathogenicity of tick-borne flaviviruses and the molecular mechanism of the flavivirus-specific antiviral activity of Oas1b, which cannot be analyzed in most inbred laboratory mice due to the lack of a functional *Oas1b* gene.

Acknowledgments This work was supported by Grants-in-Aid for Scientific Research (22780268) and the Global COE Program from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan, and Health Sciences Grants for Research on Emerging and Re-emerging Infectious Disease from the Ministry of Health, Labour and Welfare of Japan.

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特集

人と動物の共通感染症最前線　10

日本のげっ歯類におけるハンタウイルス感染の血清疫学調査とエゾヤチネズミが保有するHokkaidoウイルスの分離

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要　約

ハンタウイルスはげっ歯類媒介性の人獣共通感染症の病原体で，腎症候性出血熱（HFRS）またはハンタウイルス肺症候群（HPS）を引き起こす。近年の日本におけるげっ歯類のハンタウイルス感染状況を明らかにするために，1994年から2010年にかけて国内の様々な地域で捕獲された1658匹のげっ歯類の血清について，抗ハンタウイルス抗体の検出を行った。840例の*Rattus*属げっ歯類（ドブネズミとクマネズミ）は全て抗体陰性であった。北海道以外の地域で捕獲された野生げっ歯類113例はいずれも抗体陰性であったのに対し，北海道で捕獲された705例の野性げっ歯類のうち，エゾヤチネズミの7.4%（26/352）とアカネズミの1.2%（2/168）が抗体を保有していた。

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ハンタウイルスやフラビウイルスなどのウイルス性人獣共通感染症の病原体について研究しています。病原体の自然界での存続様式を解明するため，野外調査を行っています。調査で得られた材料の実験室内での解析を通じて，流行地や病原巣動物，病原体の性状などを明らかにしたいと思っています。大学院生募集中です。

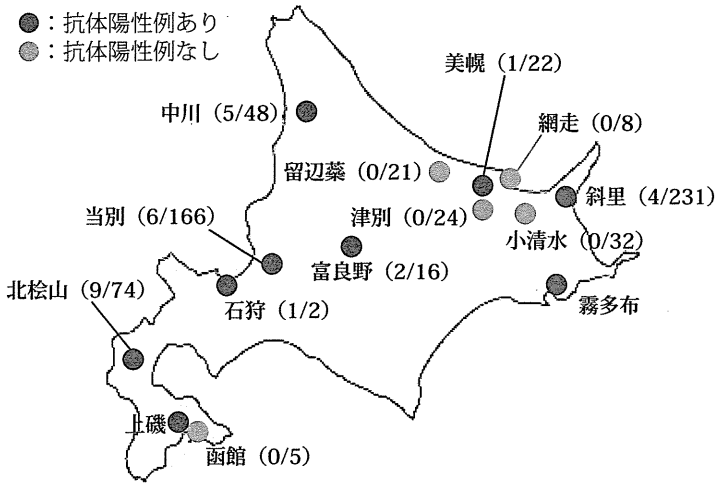
はじめに

ハンタウイルスは自然宿主であるげっ歯類と共に進化してきたと考えられており，様々なウイルスがそれぞれ固有のげっ歯類を自然宿主として世界中に分布している[3]。そのうち一部のウイルスは人に重篤なハンタウイルス感染症を引き起こすことが知られている[7]。我が国では，かつてドブネズミや実験室ラットを介したSeoul型ハンタウイルス（Seoul virus：SEOV）の感染によるHFRSの発生がみられたが[6,8]，1985年以降，人におけるハンタウイルス感染症の発生報告はない[1]。しかし，1990年代までのげっ歯類を対象とした血清疫学調査で，全国の主たる港湾地域を中心としてSEOVがドブネズミに感染していることが示唆されているほか[1,9]，北海道に生息するエゾヤチネズミ（タイリクヤチネズミの亜種）がHokkaido型のハンタウイルス（Hokkaido virus：HOKV）を保有していることが知られている[5]。これまで，HOKVによる人のハンタウイルス感染は知られておらず，本ウイルスは人に対して病原性はないものと考えられている。本研究では，近年日本国内で捕獲されたドブネズミやクマネズミなどの*Rattus*属げっ歯類と野生げっ歯類を対象としたハンタウイルス感染の血清疫学調査を実施するとともに，エゾヤチネズミからHOKVの分離を試みた。

日本のげっ歯類におけるハンタウイルスの感染状況

日本国内の20の都道府県から，合計840例の*Rattus*属げっ歯類と818例の*Rattus*属以外のげっ歯類の血清が集められ，これらの血清について，酵素抗体法（ELISA）と間接蛍光抗体法（IFA）により抗ハンタウイルス抗体の

検出を試みた。その結果，840 例の *Rattus* 属げっ歯類の血清はいずれも抗体陰性であった（北海道内で捕獲 61 例，北海道以外で捕獲 779 例）。*Rattus* 属以外の野生げっ歯類については，北海道で捕獲された 705 例のうちエゾヤチネズミの 7.4%（26/352）とアカネズミの 1.2%（2/168）が HOKV に対する抗体を保有していたのに対し，北海道以外で捕獲された 113 例はいずれも抗体陰性であった（図 1）。したがって，本州以南の日本では，げっ歯類におけるハンタウイルス感染率は極めて低いことが示唆された。一方，北海道ではエゾヤチネズミ（図 2）が HOKV を保有しており，北海道内の広範囲に亘って感染個体が検出された（図 1）。中和試験もしくはウイルス遺伝子の解析から，2 例のアカネズミ陽性例はいずれも HOKV の感染であった。



◆北海道：705 例中 28 例が抗体陽性
・エゾヤチネズミ：26/352（7.4%）　・アカネズミ：2/168（1.2%）
・その他：0/185（0.0%）
◆北海道以外：113 匹が全て抗体陰性（富山県，兵庫県，島根県）

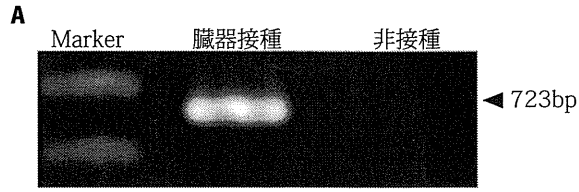
図 1　日本の野生げっ歯類におけるハンタウイルス感染の血清疫学調査

エゾヤチネズミからの Hokkaido virus の分離

エゾヤチネズミから得られた腎臓の細胞を継代培養し，培養細胞株の樹立を試みたところ，100 代以上継代しても安定した性質を持つ培養細胞株を得ることに成功した。本細胞株を MRK101 細胞と命名し，各種ハンタウイルスに対する感受性を解析した結果，*Myodes* 属のげっ歯類を宿主とする Puumala 型ハンタウイルス（Puumala virus：PUUV）に対して高い感受性を示した。そこで本細胞株を用いることにより，エゾヤチネズミ（*Myodes rufocanus bedfordiae*）を宿主とする HOKV の分離を試みた。MRK101 細胞に HOKV 感染エゾヤチネズミの肺乳剤を接種し，14 日間培養したところ，細胞中にウイルス遺伝子とウイルス抗原が検出され（図 3），培養上清中にも

写真提供：土屋公幸 博士

図 2　エゾヤチヤチネズミ（*Myodes rufocanus bedfordiae*）



HOKV 感染エゾヤチネズミの肺乳剤を MRK101 細胞に接種し，14 日目の細胞について RT-PCR によるウイルス遺伝子の検出（A）と IFA によるウイルス抗原の検出（B）を行った。

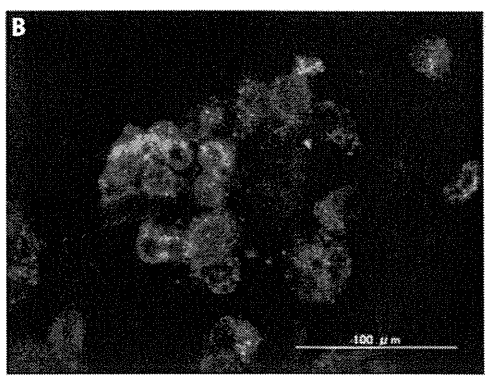


図 3　MRK101 細胞による HOKV の分離