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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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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

Abstract

Tick-borne encephalitis virus (TBEV) is a zoonotic agent that causes fatal encephalitis in humans. 2'-5'-Oligoadenylate synthetase 1b (*Oas1b*) was 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 the 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 difference of neurovirulence in the two TBEV strains.

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.

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 identity between the two strains is more than 98% [7], comparison 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 (2-5A) [22, 24]. These 2-5A 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 details.

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 37ºC 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 −80ºC. 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 an 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'-CATGAACAACAGGTGGATCCTCCACGC-3'; β-actin-reverse, 5'-CAGTTTTGGAAGTTTCTGGTAAGTCTTCG-3'; *Oas1a*-forward, 5'-TGTTAATACTTCCAGCAAGC-3'; *Oas1a*-reverse, 5'-GCAAAGACAGTGAGCAACTCT-3'; *Oas1b*-forward, 5'-AGGCTGCCGCCTGGCTGCAAT-3'; *Oas1b*-reverse, 5'-TAAGGCAGGAGGATGGCAATA-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 the *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 the 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

150 infected with serial doses of the Sofjin-HO or Oshima 5-10 strain. In B6 mice, although some mice
151 survived at low doses of infection (10 and 100 pfu), most of the mice died following intracerebral
152 infection with either TBEV strains (Table 1). All mice showing signs of illness died within 2 days
153 from the onset of the disease. The mice that survived at a low dose of infection did not show any
154 clinical signs of disease. The 50% lethal dose (LD_{50}) in B6 mice was 16.2 pfu for Sofjin-HO and
155 20.9 pfu for Oshima 5-10. In the B6.MSM-*Oas* mice infected with the Sofjin-HO strain, some mice
156 showed resistance to viral infection at low dose (LD_{50} = 148 pfu). All mice at 10 pfu of infection
157 and 60% of the mice at 100 pfu of infection survived without any signs of illness; however, 40% of
158 the mice at 100 pfu of infection and all mice at more than 1,000 pfu of infection died. In contrast,
159 most of the B6.MSM-*Oas* mice infected with 1,000 pfu or more of the Oshima 5-10 strain showed
160 general signs of illness, but all of the mice survived.

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

172

173 **Viral clearance from *Oas*-congenic mouse brain**

174 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 (Figure 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 Figure 3, the expression levels of both *Oas1a* and *Oas1b* increased from 3-6 days post-infection, and there was no difference between infection with Sofjin-HO and Oshima 5-10. Oas1a protein expression was also similar after infection of Sofjin-HO or Oshima 5-10 (Supplementary Figure 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 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 Figure 4). Perivascular cuffing was observed prominently in the

200 brain as an anti-viral response (Figure 4a-c), and a number of degenerated Purkinje cells and
201 meningitis were observed in the cerebellum (Figure 4b). In contrast, many viral antigen-positive
202 cells and inflammatory reactions were observed throughout the brain of mice infected with the
203 Sofjin-HO strain (Table 2 and Figure 4d-f). Furthermore, necrotic or degenerated neurons with
204 inflammatory cell infiltration were observed in the cerebrum and cerebellum (Figure 4d and e).
205 Compared to the B6 mice infected with Oshima 5-10 or Sofjin-HO (Figure 5 and Table 2),
206 inflammatory infiltrations were slight in B6.MSM-*Oas* mice infected with Sofjin-HO, but the
207 histopathological signs of neural degenerations and inflammation in the Sofjin-HO were similar to
208 those observed in B6 mice infected with either strain. These data suggest that B6.MSM-*Oas* mice
209 infected with the Sofjin-HO strain died due to acute neurological dysfunction throughout the brain;
210 in contrast, the mice infected with the Oshima 5-10 strain survived because the level of viral
211 replication was reduced by the anti-viral activity of *Oas1b*.
212
213 **Discussion**
214
215 In this study, we demonstrated that B6.MSM-*Oas* mice, which possess a functional *Oas1b*
216 gene, showed different responses to two different strains of TBEV. In B6.MSM-*Oas* mice infected
217 intracerebrally with the Oshima 5-10 strain, the virus was cleared and *Oas1b* was expressed. All
218 infected B6.MSM-*Oas* mice survived while most of the virus-infected B6 mice died.
219 Dose-dependent morbidity was observed during the viral clearance phase, while the clinical
220 manifestation was transient with general signs of disease, including weight loss, without any
221 neurologic signs; these general signs were considered to be associated with the host's anti-viral
222 response, including inflammation, as observed by histopathological analysis. These results are
223 consistent with those of previous studies of flavivirus-resistant mice intracerebrally infected with
224 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 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 into 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 non-specific 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 molecular mechanism of the flavivirus-specific antiviral activity of Oas1b, which cannot be analyzed in most inbred laboratory mice due to the loss of a functional *Oas1b* gene.

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References

1. Barkhash AV, Perelygin AA, Babenko VN, Myasnikova NG, Pilipenko PI, Romaschenko AG, Voevoda MI, Brinton MA (2010) Variability in the 2'-5'-oligoadenylate synthetase gene cluster is associated with human predisposition to tick-borne encephalitis virus-induced disease. The Journal of infectious diseases 202:1813-1818
2. Chebath J, Benech P, Hovanessian A, Galabru J, Revel M (1987) Four different forms of interferon-induced 2',5'-oligo(A) synthetase identified by immunoblotting in human cells. The Journal of biological chemistry 262:3852-3857
3. Chiba N, Iwasaki T, Mizutani T, Kariwa H, Kurata T, Takashima I (1999) Pathogenicity of tick-borne encephalitis virus isolated in Hokkaido, Japan in mouse model. Vaccine 17:779-787
4. Chumakov MP, Levkovich EN (1985) Russian spring summer encephalitis., The international catalog of arboviruses including certain other viruses of vertebrates
5. Ecker M, Allison SL, Meixner T, Heinz FX (1999) Sequence analysis and genetic classification of tick-borne encephalitis viruses from Europe and Asia. The Journal of general virology 80 (Pt 1):179-185
6. Elbahesh H, Jha BK, Silverman RH, Scherbik SV, Brinton MA (2011) The Flvr-encoded murine oligoadenylate synthetase 1b (Oas1b) suppresses 2-5A synthesis in intact cells. Virology 409:262-270
7. Goto A, Hayasaka D, Yoshii K, Mizutani T, Kariwa H, Takashima I (2002) Genetic and biological comparison of tick-borne encephalitis viruses from Hokkaido and far-eastern Russia. The Japanese journal of veterinary research 49:297-307
8. Hartmann R, Olsen HS, Widder S, Jorgensen R, Justesen J (1998) p59OASL, a 2'-5' oligoadenylate synthetase like protein: a novel human gene related to the 2'-5' oligoadenylate synthetase family. Nucleic acids research 26:4121-4128
9. Hayasaka D, Ivanov L, Leonova GN, Goto A, Yoshii K, Mizutani T, Kariwa H, Takashima I (2001) Distribution and characterization of tick-borne encephalitis viruses from Siberia and far-eastern Asia. The Journal of general virology 82:1319-1328
10. Hayasaka D, Nagata N, Fujii Y, Hasegawa H, Sata T, Suzuki R, Gould EA, Takashima I, Koike S (2009) Mortality following peripheral infection with tick-borne encephalitis virus results from a

combination of central nervous system pathology, systemic inflammatory and stress responses. Virology 390:139-150

11. Hovanessian AG, Laurent AG, Chebath J, Galabru J, Robert N, Svab J (1987) Identification of 69-kd and 100-kd forms of 2-5A synthetase in interferon-treated human cells by specific monoclonal antibodies. The EMBO journal 6:1273-1280

12. Kakuta S, Shibata S, Iwakura Y (2002) Genomic structure of the mouse 2',5'-oligoadenylate synthetase gene family. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 22:981-993

13. Kovalev SY, Mukhacheva TA, Kokorev VS, Belyaeva IV (2011) Tick-borne encephalitis virus: reference strain Sofjin and problem of its authenticity. Virus genes

14. Leonova GN, Pavlenko EV, Krylova NV, Belikov SI, Kondratov IG (2011) CHARACTERIZATION OF THE STRAINS OF TICK-BORNE ENCEPHALITIS VIRUS OF THE FAR-EASTERN SUBTYPE ISOLATED FROM PATIENTS WITH DIFFERENT FORMS OF INFECTION. Internatinal Union of Micribiological Societies 2011 Congress, Sapporo, Japan

15. Lim JK, Lisco A, McDermott DH, Huynh L, Ward JM, Johnson B, Johnson H, Pape J, Foster GA, Krysztof D, Follmann D, Stramer SL, Margolis LB, Murphy PM (2009) Genetic variation in OAS1 is a risk factor for initial infection with West Nile virus in man. PLoS pathogens 5:e1000321

16. Mashimo T, Lucas M, Simon-Chazottes D, Frenkiel MP, Montagutelli X, Ceccaldi PE, Deubel V, Guenet JL, Despres P (2002) A nonsense mutation in the gene encoding 2'-5'-oligoadenylate synthetase/L1 isoform is associated with West Nile virus susceptibility in laboratory mice. Proceedings of the National Academy of Sciences of the United States of America 99:11311-11316

17. Mertens E, Kajaste-Rudnitski A, Torres S, Funk A, Frenkiel MP, Iteman I, Khromykh AA, Despres P (2010) Viral determinants in the NS3 helicase and 2K peptide that promote West Nile virus resistance to antiviral action of 2',5'-oligoadenylate synthetase 1b. Virology 399:176-185

18. Moritoh K, Yamauchi H, Asano A, Yoshii K, Kariwa H, Takashima I, Isoda N, Sakoda Y, Kida H, Sasaki N, Agui T (2009) Generation of congenic mouse strains by introducing the virus-resistant genes, Mx1 and Oas1b, of feral mouse-derived inbred strain MSM/Ms into the common strain C57BL/6J. The Japanese journal of veterinary research 57:89-99

19. Nagata N, Iwata N, Hasegawa H, Fukushi S, Yokoyama M, Harashima A, Sato Y, Saijo M, Morikawa S, Sata T (2007) Participation of both host and virus factors in induction of severe acute respiratory syndrome (SARS) in F344 rats infected with SARS coronavirus. Journal of virology 81:1848-1857

20. Perelygin AA, Scherbik SV, Zhulin IB, Stockman BM, Li Y, Brinton MA (2002) Positional cloning of the murine flavivirus resistance gene. Proceedings of the National Academy of Sciences of the United States of America 99:9322-9327

21. Pulit-Penaloza JA, Scherbik SV, Brinton MA (2012) Activation of Oas1a gene expression by type

I IFN requires both STAT1 and STAT2 while only STAT2 is required for Oas1b activation. Virology 425:71-81

22. Rebouillat D, Hovanessian AG (1999) The human 2',5'-oligoadenylate synthetase family: interferon-induced proteins with unique enzymatic properties. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 19:295-308

23. Samuel CE (2001) Antiviral actions of interferons. Clinical microbiology reviews 14:778-809, table of contents

24. Sarkar SN, Ghosh A, Wang HW, Sung SS, Sen GC (1999) The nature of the catalytic domain of 2'-5'-oligoadenylate synthetases. The Journal of biological chemistry 274:25535-25542

25. Scherbik SV, Paranjape JM, Stockman BM, Silverman RH, Brinton MA (2006) RNase L plays a role in the antiviral response to West Nile virus. Journal of virology 80:2987-2999

26. Scherbik SV, Kluetzman K, Perelygin AA, Brinton MA (2007) Knock-in of the Oas1b(r) allele into a flavivirus-induced disease susceptible mouse generates the resistant phenotype. Virology 368:232-237

27. Silvia OJ, Pantelic L, Mackenzie JS, Shellam GR, Papadimitriou J, Urosevic N (2004) Virus spread, tissue inflammation and antiviral response in brains of flavivirus susceptible and resistant mice acutely infected with Murray Valley encephalitis virus. Archives of virology 149:447-464

28. Suss J (2011) Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in Europe and Asia-an overview. Ticks and tick-borne diseases 2:2-15

29. Takano A, Yoshii K, Omori-Urabe Y, Yokozawa K, Kariwa H, Takashima I (2011) Construction of a replicon and an infectious cDNA clone of the Sofjin strain of the Far-Eastern subtype of tick-borne encephalitis virus. Archives of virology 156:1931-1941

30. Takashima I, Morita K, Chiba M, Hayasaka D, Sato T, Takezawa C, Igarashi A, Kariwa H, Yoshimatsu K, Arikawa J, Hashimoto N (1997) A case of tick-borne encephalitis in Japan and isolation of the the virus. Journal of clinical microbiology 35:1943-1947

31. Yoshii K, Konno A, Goto A, Nio J, Obara M, Ueki T, Hayasaka D, Mizutani T, Kariwa H, Takashima I (2004) Single point mutation in tick-borne encephalitis virus prM protein induces a reduction of virus particle secretion. The Journal of general virology 85:3049-3058

32. Yoshii K, Mottate K, Omori-Urabe Y, Chiba Y, Seto T, Sanada T, Maeda J, Obara M, Ando S, Ito N, Sugiyama M, Sato H, Fukushima H, Kariwa H, Takashima I (2011) Epizootiological study of tick-borne encephalitis virus infection in Japan. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 73:409-412

Figure legends

Figure 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 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.

Figure 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.

Figure 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*.

Figure 4.

406 **Histopathological features in the brain of B6.MSM-*Oas* mice following intracerebral infection**
407 **at 9 days post-infection.**
408 B6.MSM-*Oas* mice were infected with 10^4 pfu of the Oshima 5-10 (A-C) or Sofjin-HO (D-F) strain.
409 TBEV antigens were detected using E protein-specific antibodies (right columns). Meningitis in the
410 cerebellum (B, arrowhead) and perivascular cuffing (arrows) were observed in the mice infected
411 with Oshima 5-10. Necrotic or degenerated neurons (asterisks) with infiltration by inflammatory
412 cells were observed in the mice infected with Sofjin-HO.
413
414 Figure 5.
415 **Histopathological features in the brain of C57BL/6 mice following intracerebral infection at 9**
416 **days post-infection.**
417 C57BL/6 mice were infected with 104 pfu of the Oshima 5-10 (a-c) or Sofjin-HO (d-f) strain.
418 TBEV antigens were detected using E protein-specific antibodies (right columns. Slight or severe
419 meningitis (b and e, arrowhead) and inflammatory infiltrations (a, c, d, e, and f, arrows) were
420 observed in the mice infected with either strain. Degenerated cells, necrotic neurons, or
421 neuronophagia (b, e, and f, asterisks) were observed mice infected with either strain.
422

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% of weight loss. No. of sick mice/no. of infected mice

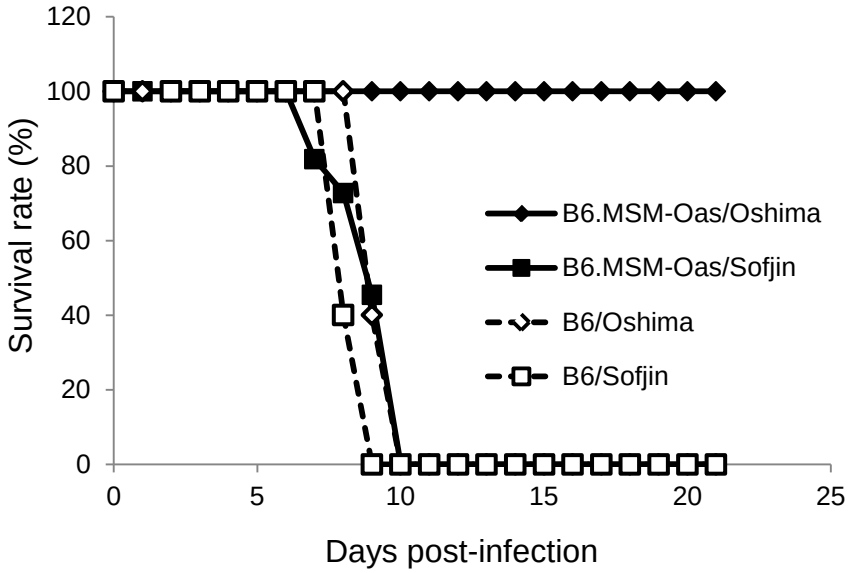
[b] No. of dead mice/no. of infected mice

Table 2. Localization of viral antigen and inflamamtory 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 (4)	0 (4)	1 (4)	0 (4)	2 (4)	0 (3)	0 (3)
	Sofjin-HO	9	4 (4)	3 (4)	4 (4)	2 (4)	4 (4)	3 (4)	2 (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)

Figure 1

a. Survival curve



b. Weight change

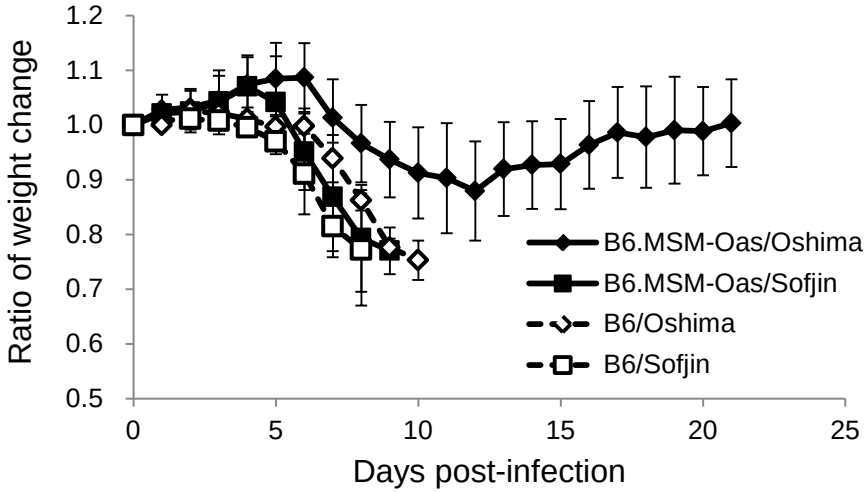


Figure 1.
Survival curves (a) and weight changes (b) following intracerebral infection with TBEV.
B6 (open symbol) and B6.MSM-*Oas* (closed symbol) mice were infected with 10^4 pfu of Sofjin-HO (diamond) or Oshima 5-10 (square) strain and monitored for 21 days. The average daily weight changes are represented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

Figure 2

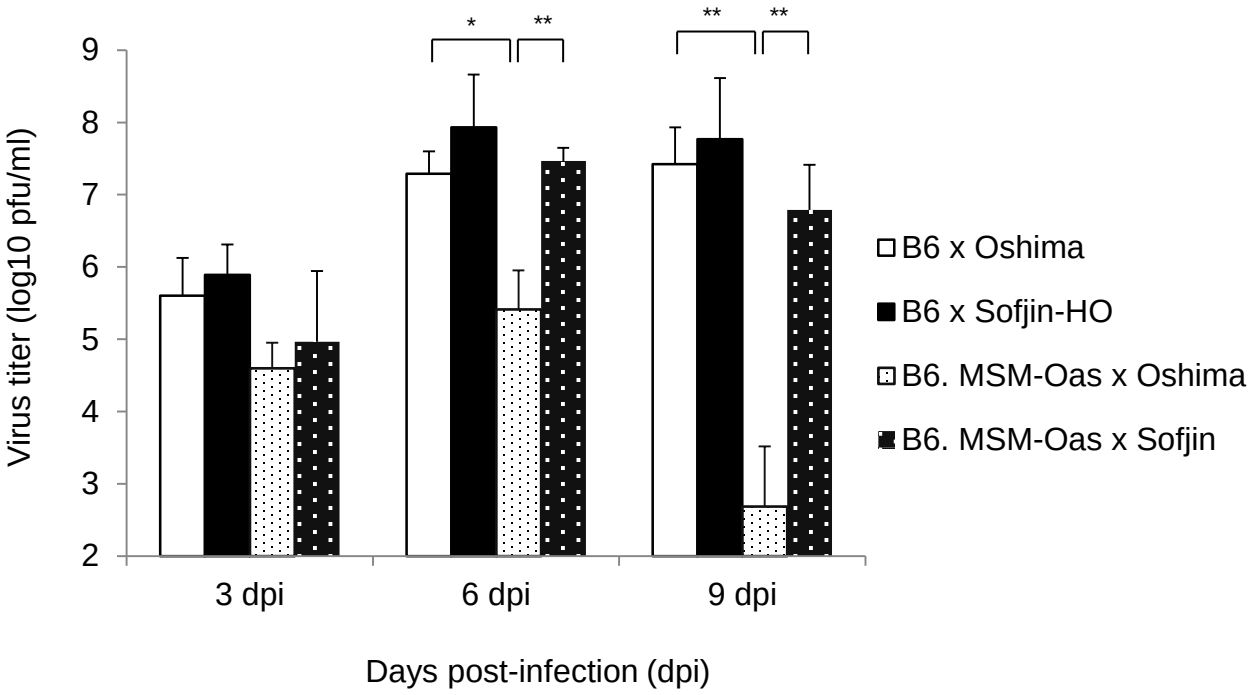


Figure 2.
Virus replication in the brain after intracerebral infection.
B6 or B6.MSM-*Oas* mice were infected with 10^4 pfu of the Sofjin-HO or Oshima 5-10 strain. Virus titers in the brain at the indicated days after infection were determined by plaque assays. Error bars represent the standard deviations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

Figure 3

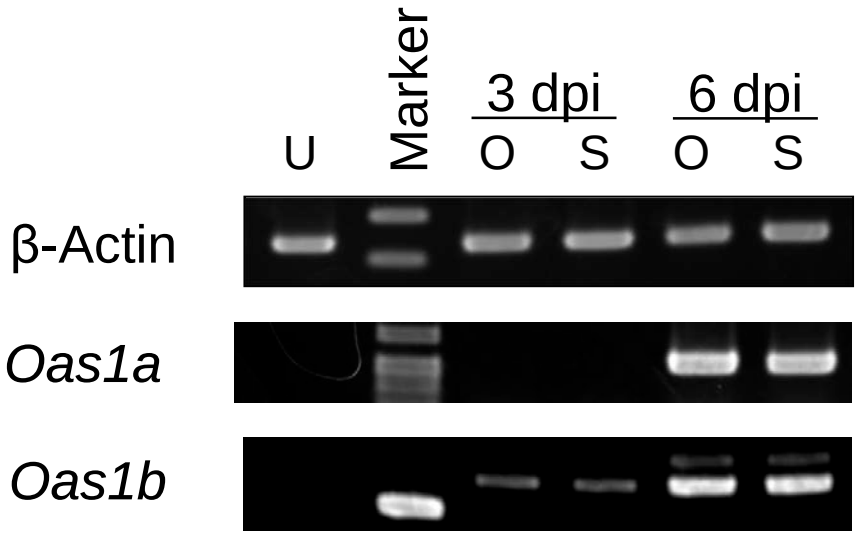


Figure 3.
Expression of *Oas* genes in the brain following intracerebral infection.
B6.MSM-*Oas* mice were infected with 10^4 pfu of the Sofjin-HO or Oshima 5-10 strain. Total brain tissue RNA (0.2 μg/reaction) from uninfected mice (U) and mice infected with the Sofjin-HO (S) or Oshima 5-10 (O) strain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

Figure 4

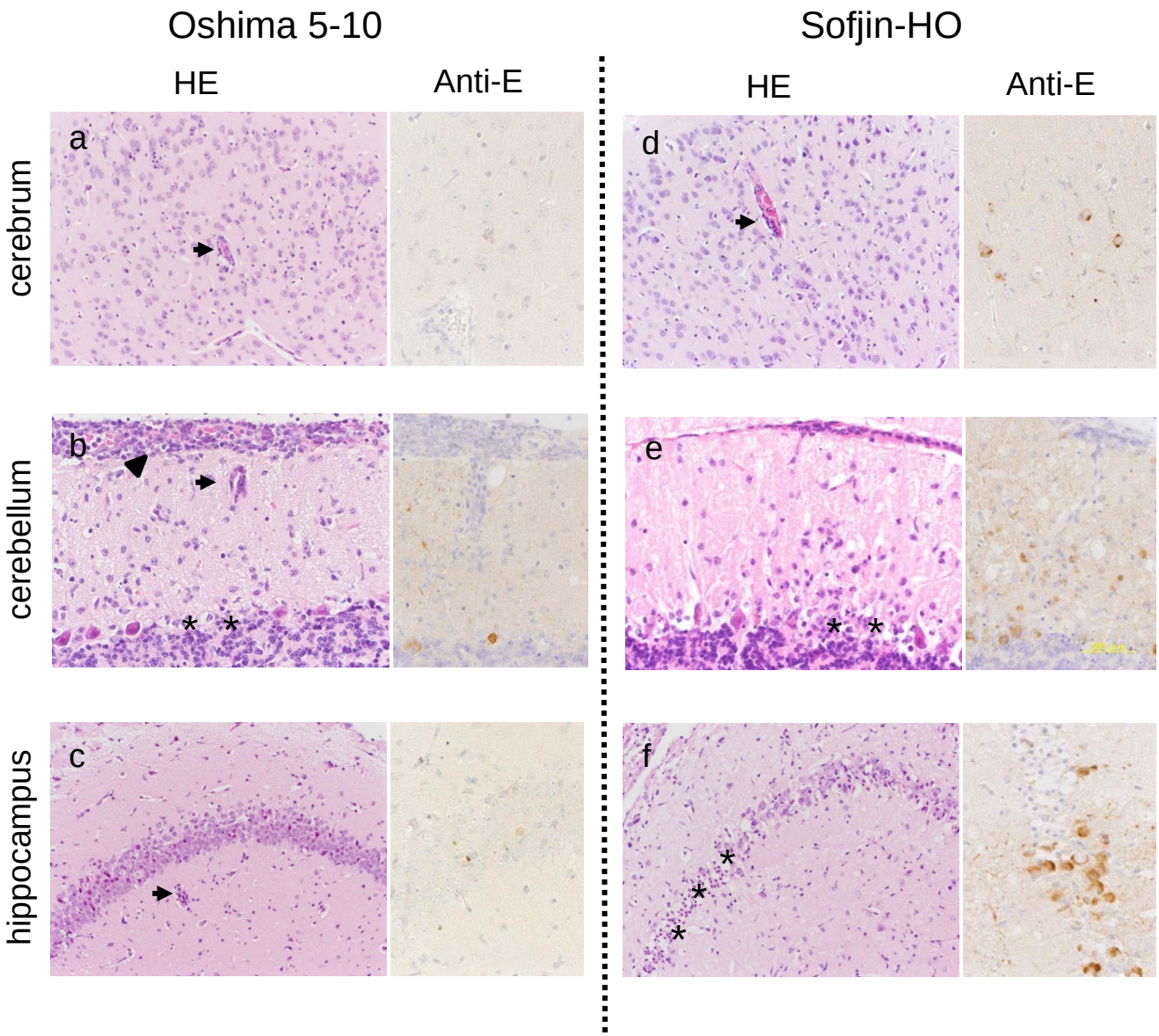


Figure 4.
Histopathological features in the brain of B6.MSM-Oas mice following intracerebral infection at 9 days post-infection.
B6.MSM-*Oas* mice were infected with 10^4 pfu of the Oshima 5-10 (a-c) or Sofjin-HO (d-f) strain. TBEV antigens were detected using E protein-specific antibodies (right columns). 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.

Figure 5

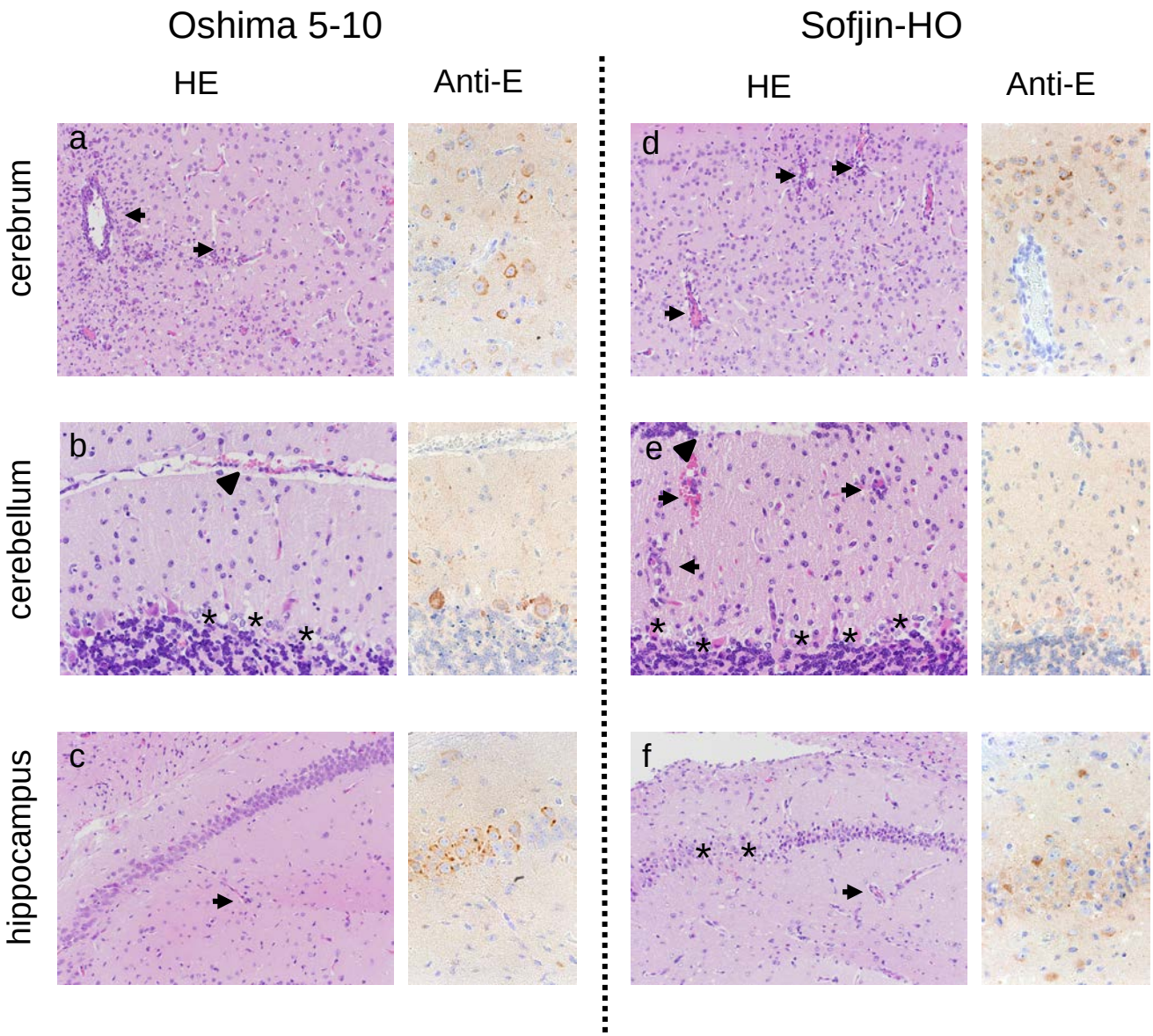
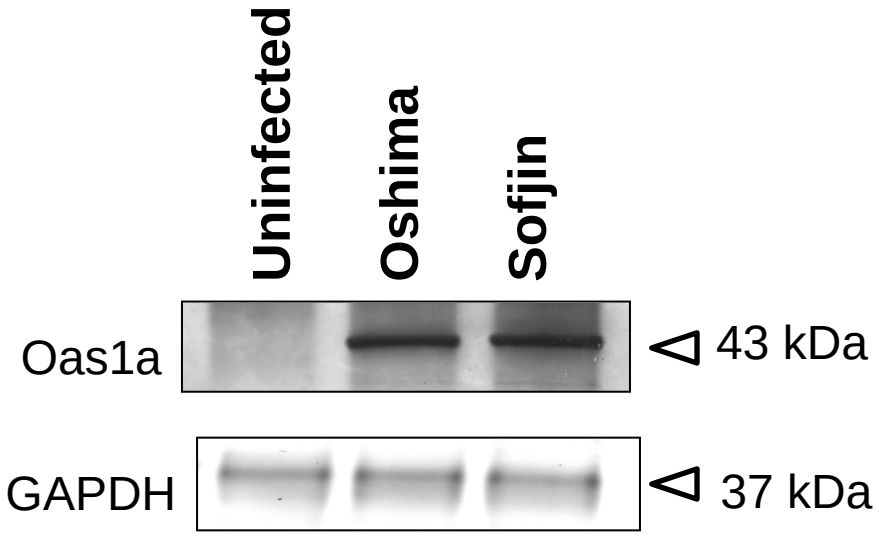


Figure 5.
Histopathological features in the brain of C57BL/6 mice following intracerebral infection at 9 days post-infection.
C57BL/6 mice were infected with 10^4 pfu of the Oshima 5-10 (a-c) or Sofjin-HO (d-f) strain. TBEV antigens were detected using E protein-specific antibodies (right columns. Slight 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.



Supplementary Figure 1.
Expression of Oas1a protein 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. brain homogenate from uninfected mice and mice infected with the Sofjin-HO or Oshima 5-10 strain at 6 days post-infection were subjected to Western blot analysis. Protein bands for Oas1a and GAPDH were detected by anti-Oas1a (Santa Cruz Biotechnology, sc-374252) and anti-GAPDH (Santa Cruz Biotechnology, sc-20357), respectively.