



Title	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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1 **Susceptibility to flavivirus-specific antiviral response of Oas1b affects the**  
2 **neurovirulence of the Far-Eastern subtype of tick-borne encephalitis virus**

3

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21 **Abbreviations**

22 BHK: Baby hamster kidney

23 B6: C57BL/6J

24 CNS: central nervous system

25 FCS: fetal calf serum

26 LD<sub>50</sub>: 50% lethal dose

27 MEM: minimum essential medium

28 OAS: 2'-5'-oligoadenylate synthetase

29 Oas1b: 2'-5'-oligoadenylate synthetase 1b

30 Pfu: plaque forming unit

31 TBE: tick-borne encephalitis

32 TBEV: tick-borne encephalitis virus

33 TBST: TBS containing 0.01% Tween 20

34 WNV: West Nile virus

35

## Abstract

36

37

38 Tick-borne encephalitis virus (TBEV) is a zoonotic agent that causes fatal encephalitis in humans.  
39 2'-5'-Oligoadenylate synthetase 1b (*Oas1b*) was identified as a flavivirus resistance gene, but most  
40 inbred laboratory mice do not possess a functional *Oas1b* gene. In this study, a congenic strain  
41 carrying a functional *Oas1b* gene, B6.MSM-*Oas*, was used to evaluate the pathogenicity of  
42 Far-Eastern TBEV. Although the intracerebral infection of B6.MSM-*Oas* mice by Oshima 5-10  
43 resulted in limited signs of illness, infection by Sofjin-HO resulted in death with severe neurologic  
44 signs. While Oshima 5-10 was cleared from the brain, Sofjin-HO was not cleared despite a similar  
45 level of expression of the intact *Oas1b* gene. Necrotic neurons with viral antigens and inflammatory  
46 reactions were observed in the brain infected with Sofjin-HO. These data indicate that the different  
47 susceptibility to the antiviral activity of *Oas1b* resulted in difference of neurovirulence in the two  
48 TBEV strains.

49

## Introduction

50

51

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

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

67 Interferon-inducible 2'-5'-oligoadenylate synthetases (OASs) play important roles in the  
68 antiviral activity against RNA virus infections. After activation by double-stranded RNA, OAS  
69 proteins polymerize adenosine 5'-triphosphate (ATP) into 2'-5'-linked oligoadenylates (2-5A) [22,  
70 24]. These 2-5A activate RNase L, resulting in the degradation of viral RNA [23]. The OAS family  
71 consists of *OAS1*, *OAS2*, *OAS3*, and multiple *OAS-like* genes in humans [2, 8, 11], and eight small  
72 *Oas1* (*Oas1a-h*), one *Oas2*, one *Oas3*, and two *Oas-like* (*OasL1* and *OasL2*) genes in mice [12].

73 The murine isoform *Oas1b* has been identified as a critical determinant of the genetic  
74 susceptibility of mice to infection with West Nile virus (WNV), a mosquito-borne flavivirus [16,

75 20]. It was recently reported that genetic variation in human OAS is associated with a predisposition  
76 to TBEV- and WNV-induced diseases [1, 15]. However, little is known about the detailed  
77 mechanism of OASs in the pathogenesis of tick-borne flaviviruses. Since most inbred laboratory  
78 mice do not possess a functional *Oas1b* gene due to a premature stop codon, the susceptibility to  
79 flaviviruses is increased. The increased susceptibility has made it difficult to analyze the roles of  
80 OASs in flavivirus pathogenesis in details.

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

87

88

## Materials and Methods

89

### *Viruses*

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

99

100 ***Virus infection in mice***

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

110

111

112 ***Viral titration***

113 Brain samples were weighed, homogenized, and prepared as 10% suspensions (w/v) in  
114 PBS supplemented with 10% FCS. The suspensions were clarified by centrifugation (4,000 rpm for  
115 5 min at  $4^{\circ}\text{C}$ ), and the viruses in the supernatants were titrated.

116 Plaque assays were performed with BHK cells using 12-well plates. Serial tenfold dilutions  
117 of the organ suspensions were inoculated to the monolayer of cells. After incubation for 1 h at  $37^{\circ}\text{C}$ ,  
118 1.5% carboxymethylcellulose-MEM was added to the cells. The incubation was continued for four  
119 days, and the monolayers were stained with 0.1% crystal violet in 10% formalin neutral buffer  
120 solution. Plaques were counted and infectivity titers were expressed as pfu/mL.

121

122 ***Semi-quantitative RT-PCR***

123 Total brain tissue RNA isolated by Isogen (Nippon Gene, Tokyo, Japan) was used for  
124 RT-PCR. Equal amounts (0.2  $\mu\text{g}$ ) of RNA were subjected to reverse transcription using SuperScript

125 II and an Oligo(dT)<sub>20</sub> Primer (Life Technologies, Carlsbad, CA, USA) at 42°C for 50 min and 70°C  
126 for 15 min, followed by 26 ( $\beta$ -actin) and 35 (*Oas1a* and *Oas1b*) PCR cycles consisting of 94°C for  
127 30 s, 55°C for 30 s and 68°C for 2 min using Platinum *Taq* (Life Technologies). The following  
128 primers were used:  $\beta$ -actin-forward, 5'-CATGAACAACAGGTGGATCCTCCACGC-3';  $\beta$ -actin-reverse,  
129 5'-CAGTTTTGGAAGTTTCTGGTAAGTCTTCG-3'; *Oas1a*-forward,  
130 5'-TGTTAATACTTCCAGCAAGC-3'; *Oas1a*-reverse, 5'-GCAAAGACAGTGAGCAACTCT-3';  
131 *Oas1b*-forward, 5'-AGGCTGCCGCCTGGCTGCAAT-3'; *Oas1b*-reverse,  
132 5'-TAAGGCAGGAGGATGGCAATA-3'.

133

134

### 135 ***Histopathological examination***

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

140

141

## 141 **Results**

142

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

144 Initially, B6.MSM-*Oas* mice were subcutaneously infected with the Sofjin-HO or Oshima  
145 5-10 strain of TBEV. Following infection with 10<sup>6</sup> pfu of either strain, all mice survived without  
146 any clinical signs. No viremia or viral multiplication in organs (spleen, lung, liver, and brain) was  
147 observed (data not shown). These data indicate that, in the B6.MSM-*Oas* mice, the virus was  
148 eliminated in the early stage of infection following subcutaneous challenge.

149 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<sub>50</sub>) 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<sub>50</sub> = 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<sup>4</sup> 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*

175 gene, viral loads in the brain were investigated after intracerebral infection with  $10^4$  pfu of TBEV.  
176 At 3 days after infection, there were no significant differences in the viral loads between the B6 and  
177 B6.MSM-*Oas* mice and between the Sofjin-HO and Oshima 5-10 strains (Figure 2). At 6 days  
178 post-infection, a lower titer of virus was observed in the B6.MSM-*Oas* mice infected with the  
179 Oshima 5-10 strain than in the B6 mice, while similarly high levels of virus ( $>10^7$  pfu/mL) were  
180 detected in both the B6 and B6.MSM-*Oas* mice infected with Sofjin-HO. This trend was more  
181 apparent at 9 days post-infection. The viral loads in the B6.MSM-*Oas* mice infected with the  
182 Oshima 5-10 strain were drastically reduced, and the virus was cleared from the brain in two of the  
183 four mice. However, the viral loads in the B6.MSM-*Oas* mice infected with the Sofjin-HO strain  
184 remained as high as in the B6 mice.

185 To examine whether the difference in viral clearance in the B6.MSM-*Oas* mice between  
186 the Sofjin-HO and Oshima 5-10 strains was due to the expression level of *Oas1b*, the expression of  
187 *Oas1a* and *Oas1b* was analyzed by semi-quantitative RT-PCR. As shown in Figure 3, the expression  
188 levels of both *Oas1a* and *Oas1b* increased from 3-6 days post-infection, and there was no difference  
189 between infection with Sofjin-HO and Oshima 5-10. *Oas1a* protein expression was also similar after  
190 infection of Sofjin-HO or Oshima 5-10 (Supplementary Figure 1). These data indicated that both  
191 the Sofjin-HO and Oshima 5-10 strains possess a comparable ability to induce *Oas1b* expression.  
192 However, the Oshima 5-10 strain was cleared from the brain as intact *Oas1b* was expressed,  
193 whereas the Sofjin-HO strain was not cleared despite a similar level of expression of intact *Oas1b*.

194

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

196 The histopathological features of the B6.MSM-*Oas* mice were examined following  
197 intracerebral infection with Sofjin-HO or Oshima 5-10 strain at 9 days post-infection. In the  
198 B6.MSM-*Oas* mice infected with Oshima 5-10, viral antigen-positive cells were rarely observed  
199 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].

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

234 The pathogenicity of TBEV in B6.MSM-*Oas* mice closely resembled that observed in  
235 human cases. The Sofjin-HO strain was derived from the brain of a TBE patient [4] and is closely  
236 related to another reference strain, Khabarovsk-Obor-4 (Accession No. FJ214111), which was also  
237 isolated from the brain of a TBE patient with a lethal outcome [13]. The Oshima 5-10 strain was  
238 isolated from a sentinel dog in Hokkaido, Japan [30]. Endemic foci of Oshima-related strains have  
239 been maintained for more than 10 years in the area [32], but only one human case of TBE has been  
240 reported. Therefore, Japanese TBEV isolates, including the Oshima 5-10 strain, have been  
241 considered to be relatively-avirulent. A recent phylogenetic analysis of the Far-Eastern subtype of  
242 TBEV revealed that the Sofjin-HO strain is genetically closely related to strains isolated from TBE  
243 patients in Far-Eastern Russia, whereas the Oshima 5-10 strain is more closely related to strains  
244 isolated from asymptomatic patients bitten by ticks [14]. Our results concerning the pathological  
245 features of intracerebral infection in B6.MSM-*Oas* mice, which were not identified in B6 mice,  
246 agreed with the genetic background of the strains. Peripheral infection in B6.MSM-*Oas* mice  
247 resulted in viral clearance during the initial stage of infection, and the mice showed no signs of  
248 disease. This is consistent with previous data for WNV [18], and may be related to the low  
249 incidence rate in flavivirus-infected individuals. Our previous data showed that peripheral viral

250 multiplication did not differ between the Sofjin-HO and Oshima 5-10 strains in B6 mice and did not  
251 correlate with the progression of disease in TBEV infection [10, 29]. Considering the results of  
252 intracerebral infection in B6.MSM-*Oas* mice, it may be possible to distinguish clinical conditions  
253 based on the TBEV strain after viral invasion into the brain.

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

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

273

274

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380

381

382 **Figure legends**

383

384 Figure 1.

385 **Survival curves (A) and weight changes (B) following intracerebral infection with TBEV.**

386 B6 (open symbol) and B6.MSM-*Oas* (closed symbol) mice were infected with  $10^4$  pfu of Sofjin-HO  
387 (diamond) or Oshima 5-10 (square) strain and monitored for 21 days. The average daily weight  
388 changes are represented as a ratio to the weight at day 0. Error bars represent the standard  
389 deviations.

390

391 Figure 2.

392 **Virus replication in the brain after intracerebral infection.**

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

397

398 Figure 3.

399 **Expression of *Oas* genes in the brain following intracerebral infection.**

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

404

405 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  $10^4$  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 <sup>a</sup>		mortality <sup>b</sup>		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 <sub>50</sub> (pfu)	148				-				16.2				20.9			

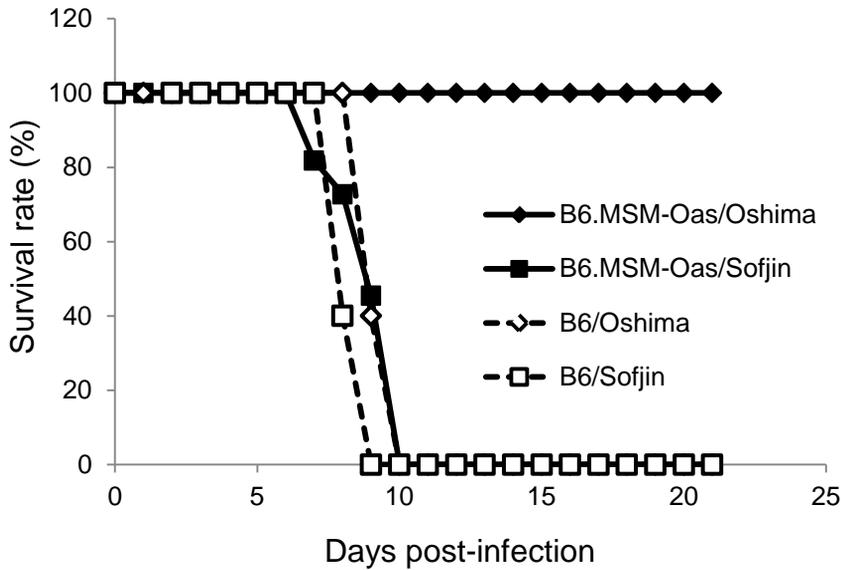
<sup>a</sup> Morbidity of mice was estimated by >10% of weight loss. No. of sick mice/no. of infected mice

<sup>b</sup> No. of dead mice/no. of infected mice



# Figure 1

a. Survival curve



b. Weight change

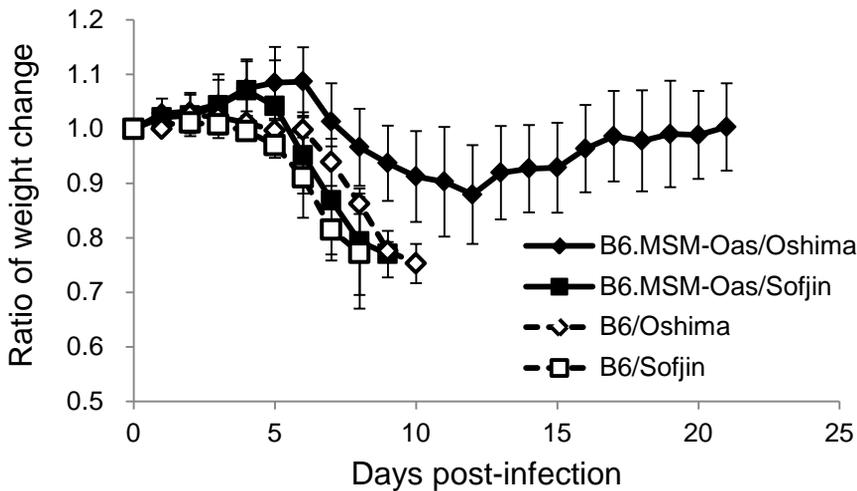


Figure 1.

**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

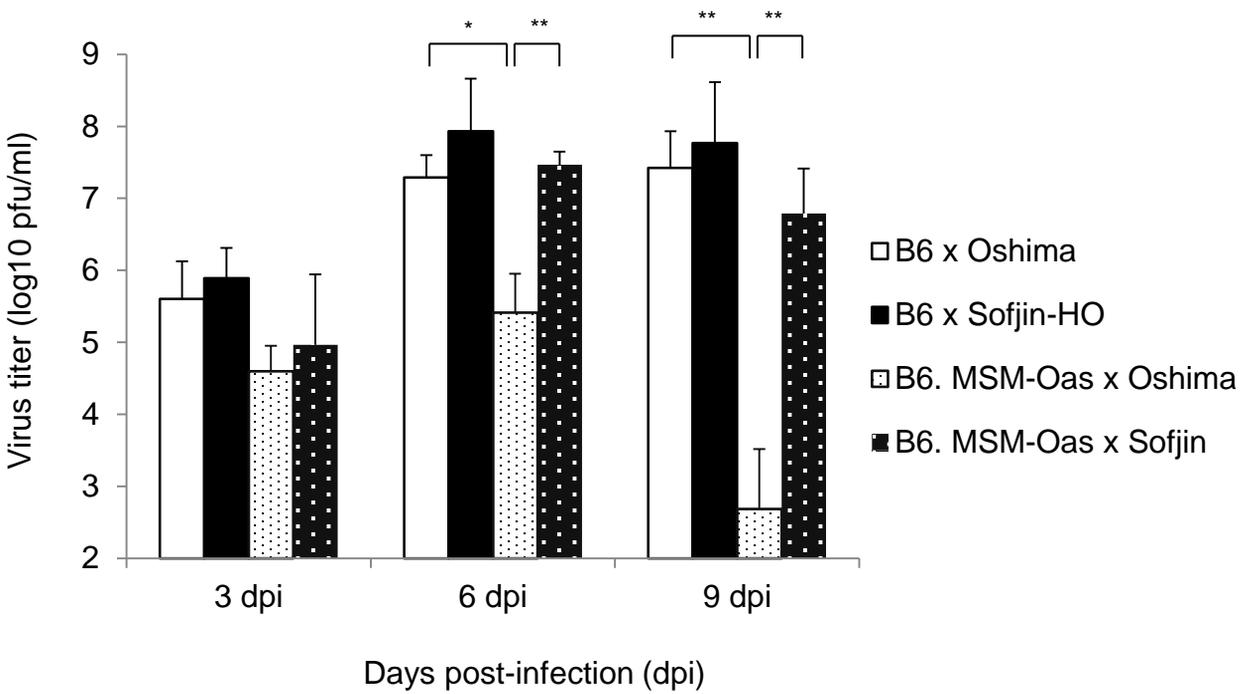


Figure 2.  
**Virus replication in the brain after intracerebral infection.**  
B6 or B6.MSM-Oas mice were infected with 10<sup>4</sup> pfu of the Sofjin-HO or Oshima 5-10 strain. Virus titers in the brain at the indicated days after infection were determined by plaque assays. Error bars represent the 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

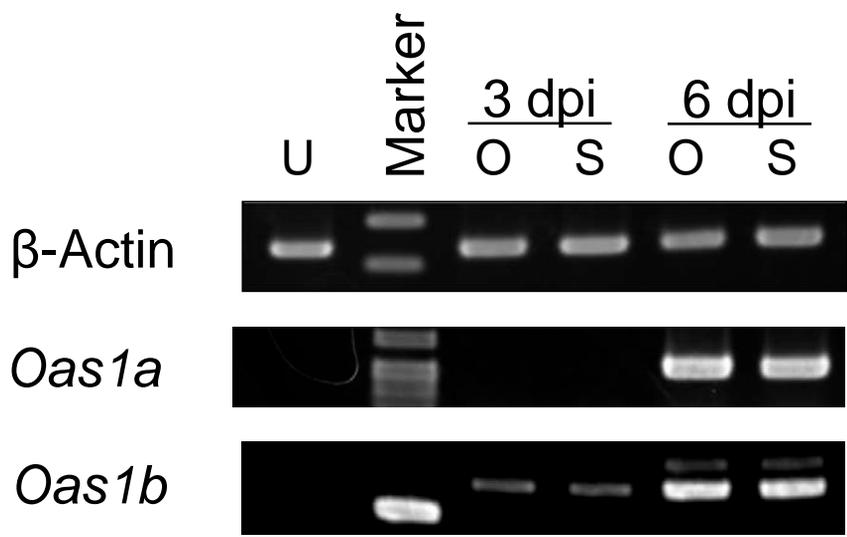


Figure 3.

**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 ( $0.2 \mu\text{g}/\text{reaction}$ ) 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  $\beta$ -actin, *Oas1a*, and *Oas1b*.

# Figure 4

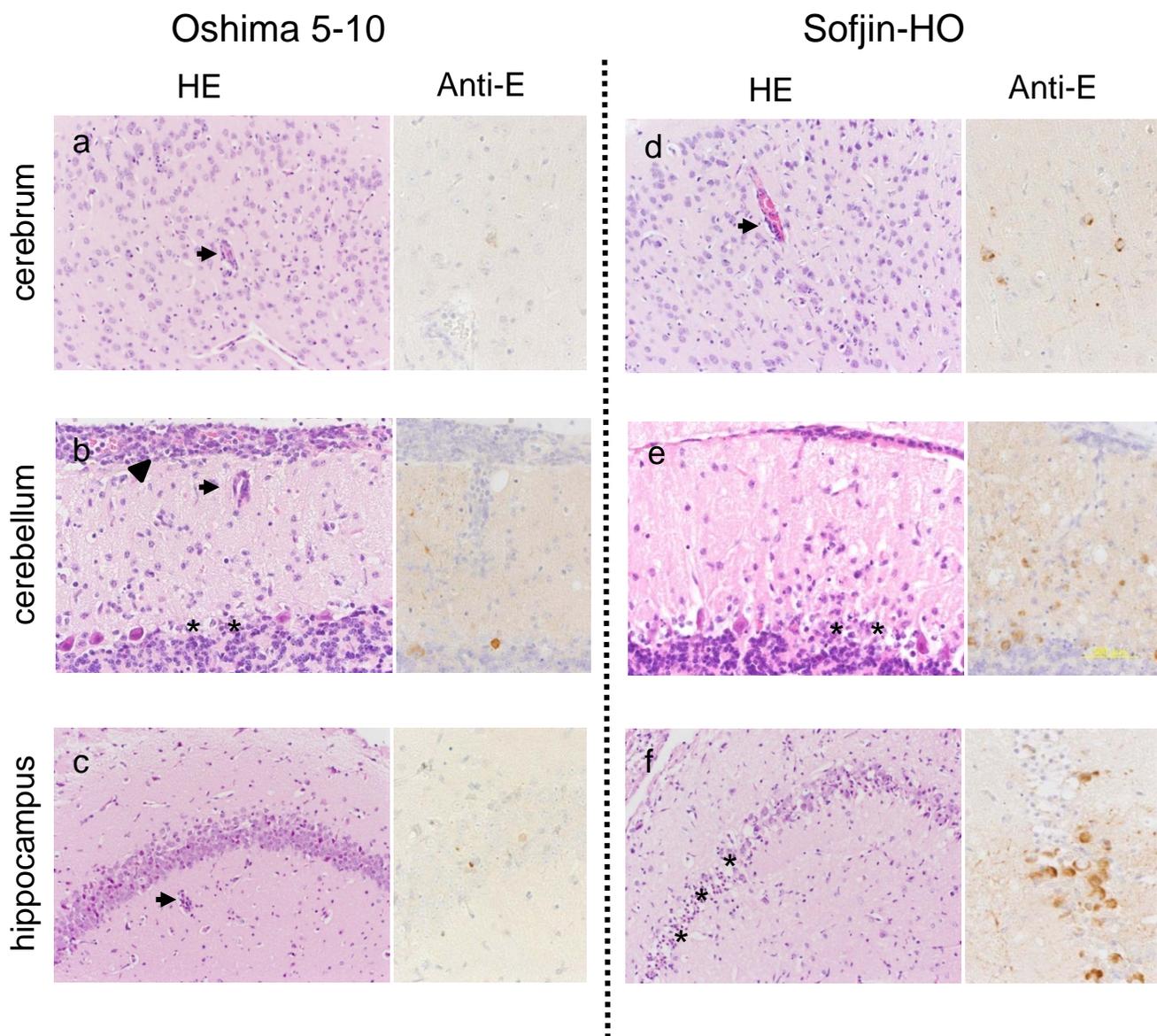


Figure 4.  
**Histopathological features in the brain of B6.MSM-Oas mice following intracerebral infection at 9 days post-infection.**  
B6.MSM-Oas mice were infected with  $10^4$  pfu of the Oshima 5-10 (a-c) or Sofjin-HO (d-f) strain. TBEV antigens were detected using E protein-specific antibodies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

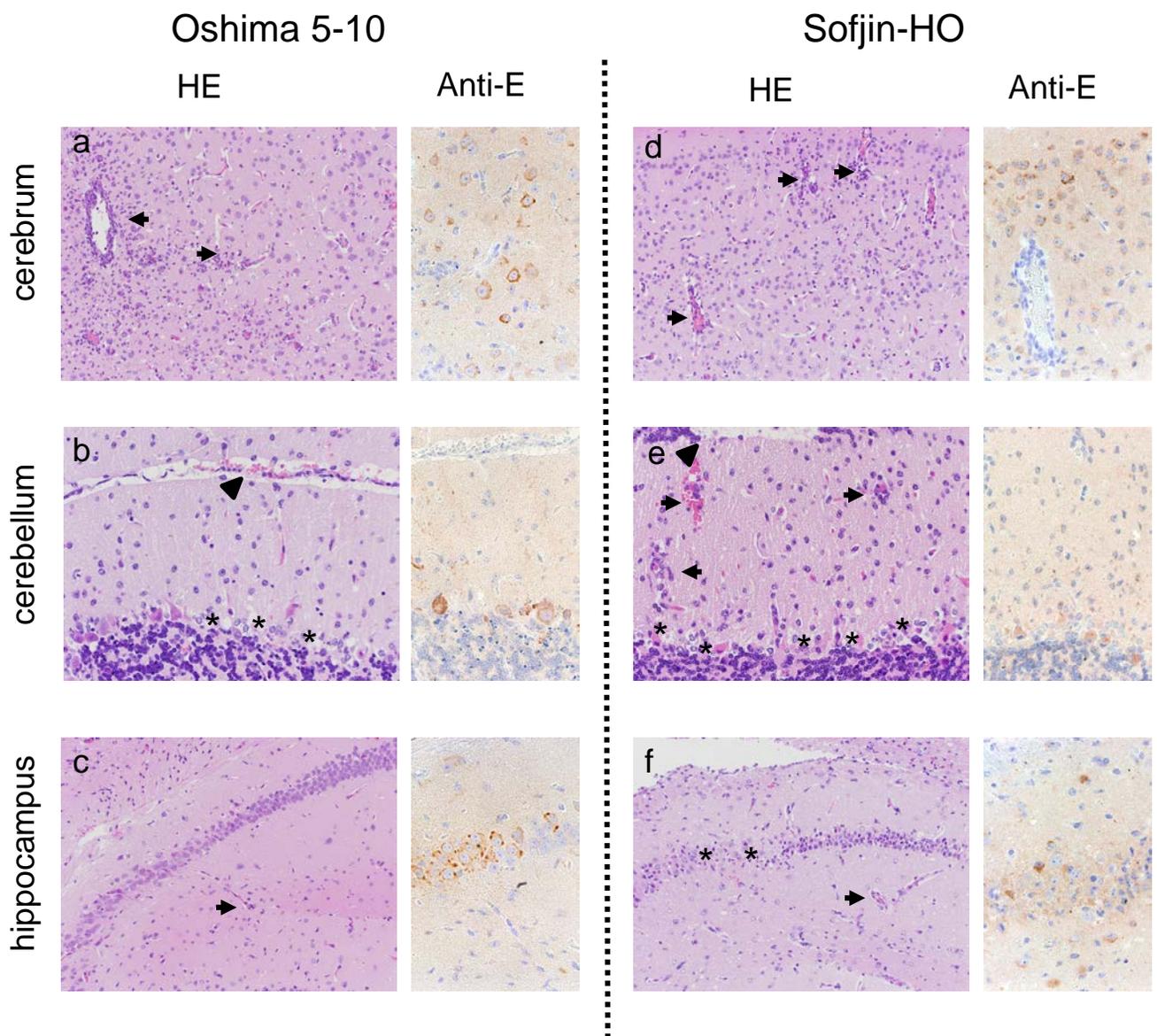
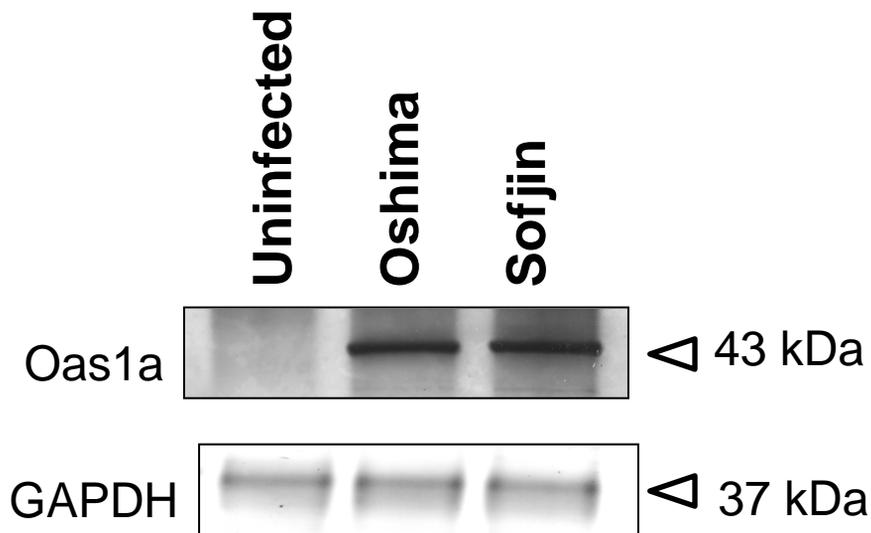


Figure 5.  
**Histopathological features in the brain of C57BL/6 mice following intracerebral infection at 9 days post-infection.**

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.