Comparison of the antiviral potential among soluble forms of herpes simplex virus type-2 glycoprotein D receptors, herpes virus entry mediator A, nectin-1 and nectin-2, in transgenic mice

Yoshikazu Fujimoto,1,2,* Yukiko Tomioka,3 Kinuyo Ozaki,2 Keiko Takeda,2 Haruka Suyama,2 Sayo Yamamoto,2 Hiroki Takakuwa,4 Masami Morimatsu,5 Toshimitsu Uede6 and Etsuro Ono1,2,*

Abstract
Herpesvirus entry mediator A (HVEM), nectin-1 and nectin-2 are cellular receptors of glycoprotein D (gD) of herpes simplex virus type-2 (HSV-2). It has been shown that soluble forms of HSV gD receptors have the antiviral potential in cultured cells and transgenic mice. Here, to compare antiviral potential of soluble forms of HVEM, nectin-1 and nectin-2 against HSV-2 infections in vivo, transgenic mice expressing fusion proteins consisting of the entire ectodomain of HVEM, nectin-1 or nectin-2 and the Fc portion of human IgG (HVEMIg, nectin-1Ig and nectin-2Ig, respectively) were intraperitoneally infected with HSV-2. In the infection with 3 MLD50 (50 % mouse lethal dose), effective resistance was not observed in transgenic mice expressing nectin-2Ig. In a transgenic mouse line with high expression of nectin-1Ig, significant protection from the infection with 30 and 300 MLD50 was observed (survival rate of 100 and 71 %, respectively). On the other hand, transgenic mice expressing HVEMIg showed a complete resistance to the lethal infection even with 300 MLD50 (survival rate of 100 %). These results demonstrated that HVEMIg could exert effective antiviral activities against HSV-2 infections in vivo as compared with other soluble forms of HSV gD receptors.

INTRODUCTION
Herpes simplex virus (HSV), a representative member of the subfamily Alphaherpesvirinae, commonly infects the skin, eyes and the mucous membrane in regions of the mouth and genitalia [1]. Genital herpes is a common sexually transmitted disease that is caused by herpes simplex virus type-2 (HSV-2) [2].

HSV-2 initiates host epithelial cell infection through a sequential set of viral envelope glycoprotein–host cell surface interactions. First, HSV-2 glycoproteins B and C (gB and gC) bind to host cell heparan sulfate. Second, the viral glycoprotein D (gD) protein binds one of several host coreceptors. Third, the HSV glycoprotein H/L (gH/L) complex promotes viral envelope–host cell plasma membrane fusion, allowing capsid entry [1]. Receptors of gD have been identified: herpesvirus entry mediator A (HVEM), heparin sulfate generated by the action of specific 3-O-sulfotransferases, nectin-1 and nectin-2 [1]. Nectin-1 and HVEM are competent receptors for both HSV-1 and HSV-2 entry, while nectin-2 is a receptor for HSV-2 and not for wild-type of HSV-1 [3–5]. Taylor et al. [6] reported that lethal HSV-2 infections via the vaginal route were completely prevented in HVEM/nectin-1 double-knockout mice, but not in either HVEM or nectin-1 KO mice, demonstrating that both HVEM and nectin-1 were responsible for HSV-2 infection in vivo. On the other hand, although it has been demonstrated that expressing nectin-2 in various human cell types of neuronal, fibroblastic and epithelial origin is able to mediate the entry of HSV-2 into cultured cell lines [4, 5], an in vivo role of nectin-2 for the viral infection is not clearly understood.
There is no cure for genital herpes, but medication is available to ease symptoms and reduce the risk of infecting others. Three antiviral medications provide clinical benefit for genital herpes: acyclovir, valacyclovir and famciclovir. However, HSV-2 mutant strains that are resistant to these antiviral medications have been isolated. All acyclovir-resistant strains are also resistant to valacyclovir, and most are resistant to famciclovir. In addition, there is currently no commercially available vaccine that is protective against genital herpes infection. It has been shown that soluble forms of gD receptors effectively inhibited viral entry of HSV-1 [7, 8], and HSV-2 propagation into cultured cells [9]. Previously, we also demonstrated that transgenic mice expressing the soluble form of murine HVEM and porcine nectin-1 showed marked resistance to lethal infections with HSV-1 [10, 11]. To date, there is no comparative study on antiviral potential among soluble forms of gD receptors against HSV-2 infections in vivo. Although it has yet to be definitively determined whether porcine nectin-1 acts as an entry receptor for HSV-2, viral entry activity of human nectin-1 has been demonstrated [12]. Human nectin-2 can also serve as an entry receptor for HSV-2, but not murine nectin-2 [4], whereas it was reported that activity of HSV-2 cell entry mediated by human HVEM was indistinguishable from that of murine HVEM [12]. In the present study, we have for the first time demonstrated the anti-HSV-2 potential of the soluble form of gD receptors in vivo through experimental infection in transgenic mice expressing soluble forms of human nectin-1 (nectin-1Ig) and human nectin-2 (nectin-2Ig), and murine HVEM (HVEMIg).

RESULTS

In previous studies, we have already established transgenic mouse lines expressing murine HVEM Ig [10] and human nectin-2Ig [13]. To compare the resistance of transgenic mouse lines expressing HVEMIg, nectin-1Ig and nectin-2Ig against HSV-2 infection, we initially generated transgenic mouse lines expressing human nectin-1Ig, which was demonstrated to effectively inhibit HSV-2 infection in vitro (Fig. S1, available in the online Supplementary Material). All of the transgenes consisting of the extracellular domain of each gD receptor fused to the Fc region of human IgG1 are expressed under the control of the CAG promoter. Three transgenic mouse lines expressing nectin-1Ig (2695, 2708 and 6473) were generated. Expression of nectin-1Ig in the transgenic mouse sera was confirmed by Western blot analyses using anti-human IgG antibody (Fig. 1a). As reported in the other transgenic mouse lines [10, 13], expression of nectin-1Ig was also observed in various organ tissues by immunohistochemical analyses (Fig. S2). In addition to transgenic mouse lines expressing nectin-1Ig, expression of each transgene in sera of nectin-2Ig (lines 2216 and 2246) and HVEMIg (lines A and C) transgenic mice was confirmed by Western blot analyses using anti-human IgG antibody (Fig. 1a). Each band of nectin-1Ig, nectin-2Ig and HVEMIg was also detectable by Western blot analyses using specific antibodies to human nectin-1, human nectin-2 and murine HVEM, respectively (Fig. 1a). To compare expression levels of each fusion protein in transgenic mouse sera, a competitive ELISA was performed (Fig. 1b). Average concentrations of nectin-1Ig in serum samples from five transgenic mice per line were 30.7 µg ml⁻¹ for line 2695, 215.2 µg ml⁻¹ for line 2708 and 90.2 µg ml⁻¹ for line 6473, indicating that expression levels of the fusion protein are distinctly different between transgenic mouse lines. On the other hand, differences in expression levels of each nectin-2Ig (939.4 µg ml⁻¹ for line 2216 and 900.3 µg ml⁻¹ for line 2246) and HVEMIg (246.9 µg ml⁻¹ for line A and 390.8 µg ml⁻¹ for line C) are insignificantly small between transgenic mouse lines.

We previously demonstrated that transgenic mice expressing porcine nectin-1Ig showed remarkable resistance to HSV-1 infection [11]. Because transgenic mice expressing human nectin-1Ig were newly generated in the present study, experimental infection with 3 MLD₅₀ (50 % mouse lethal dose) of HSV-1 strain VR3 was conducted to confirm the resistance against HSV-1 infection. At day 14 post-infection, transgenic mouse lines 6473 and 2708 showed significant resistance: survival rates were 100 and 86 %, respectively (Fig. 2a). On the other hand, only 13 % of mice of line 2695 survived after the infection with HSV-1. The results that mouse lines which effectively expressed nectin-1Ig showed significant resistance indicates that the difference in survival rates between these three lines was dependent on the expression level of nectin-1Ig. According to our previous report [10], all the transgenic mice expressing HVEMIg survived after infection with HSV-1 strain VR3 at 3 MLD₅₀ (Fig. 2a). These results demonstrated that transgenic mice expressing human nectin-1Ig and HVEMIg used in this study had antiviral potential against HSV-1.

To examine resistance of the transgenic mice expressing HVEMIg, nectin-1Ig and nectin-2Ig against HSV-2 infection, experimental infections with 3 MLD₅₀ of strain 186, which showed high pathogenicity in mice, were conducted. Survival data until 21 days post-inoculation (d p.i.) are shown in Fig. 3(a). Approximately 95 % of all non-transgenic littermates used as a control were dead by 15 d p.i. In contrast, both lines of transgenic mice expressing HVEMIg showed complete resistance (survival rate 100 %). In transgenic mice expressing nectin-1Ig, all the mice of line 6473 and half of line 2708 survived (survival rate 50 %), but almost all of the mice of line 2695 died (survival rate 15 %). In contrast, 100 and 80 % of the transgenic mice expressing nectin-2Ig, lines 2216 and 2246, respectively, died in the HSV-2 infection, although the expression level of nectin-2Ig was as effective as those of HVEMIg and nectin-1Ig (Fig. 1b), demonstrating that nectin-2Ig did not show the antiviral effect against HSV-2 infections in vivo. Subsequently, to further investigate the antiviral potential of HVEMIg and nectin-1Ig against HSV-2 challenges with higher infective doses, the transgenic mice expressing nectin-1Ig (lines 2708 and 6473) and HVEMIg (lines A and C) were inoculated with strain 186 at 30 and 300 MLD₅₀. In the viral challenge with 30 MLD₅₀, almost all of transgenic
mice line 2708 expressing nectin-1Ig and non-transgenic littermates succumbed (Fig. 3b). In contrast, all the mice of HVEMIg lines A and C, and of nectin-1Ig line 6473 survived (Fig. 3b). When the mice of HVEMIg line A and C were inoculated with 300 MLD_{50}, almost all of the mice survived without any clinical symptoms during the experimental period (Fig. 3c). On the other hand, 71% of the mice of line 6473 survived and several mice succumbed (Fig. 3c), although the expression level of the fusion protein in line 6473 was higher than those in HVEMIg lines A and C (Fig. 1b). These results demonstrated that HVEMIg could effectively protect transgenic mice from HSV-2 lethal infections, and the antiviral potential of nectin-1Ig was weaker than that of HVEMIg in vivo.

It has been reported that soluble forms of gD receptors inhibited cell entry of HSV-1 in cultured cells [7, 8, 14–18]. To examine the antiviral activity of soluble forms of gD receptors in sera of transgenic mice of HVEMIg lines A and C, nectin-1Ig lines 2708 and 6473, and nectin-2Ig line 2216, neutralization assays were performed using HSV-2 strains 186, YS-4 and 8620 KN. As expected, it was found that transgenic mouse serum containing nectin-1Ig significantly inhibited infections with all tested HSV-2 strains in Vero cells (Table 1), suggesting that one of the mechanisms of resistance to HSV-2 infection observed in transgenic mouse lines 2708 and 6473 was the viral neutralizing effect of nectin-1Ig. Consistent with an earlier study [9], the serum containing nectin-2Ig did not show any neutralization activity. Surprisingly, contrary to expectation, the number of viral plaques was not decreased by the serum containing HVEMIg (Table 1), although transgenic mice expressing HVEMIg were highly resistant to lethal infections with HSV-2 (Fig. 3). In addition, to demonstrate whether purified nectin-1Ig, nectin-2Ig and HVEMIg show neutralizing activity against HSV-2 infection, transgenic mouse sera were used for affinity chromatography with protein G-Sepharose 4B, and 800 µg ml^{-1} of each fusion protein was prepared. Consistent with the neutralizing assay using

![Fig. 1. Expression of nectin-1Ig, nectin-2Ig and HVEMIg in sera of transgenic mice. (a) Western blot analysis of sera from the transgenic mice. (b) Quantification of expression level of fusion proteins was done by ELISA. The mean concentrations (µg ml^{-1}) of the protein from five mice per transgenic line are shown. Error bars indicate ±sd.](image-url)
transgenic mouse sera, purified nectin-1Ig showed effective reduction of HSV-2 plaque numbers but purified nectin-2Ig and HVEMIg did not (data not shown).

Furthermore, ELISA and neutralization assays were performed to determine whether anti-HSV-2 antibodies could be detected in sera from the mice intraperitoneally inoculated with strain 186. All sera collected from surviving mice reacted with recombinant gD of strain 186 in ELISA, and those sera of HVEMIg lines A and C decreased viral plaque formation in the cultured cells in a neutralization assay (Table 1). These findings suggest that HSV-2 infection was established in the transgenic mice expressing HVEMIg, and antibodies against HSV-2 were produced in the mice. Taken together, these findings indicated that complete protection observed in transgenic mice expressing HVEMIg was not the predominant mechanism, which is caused by neutralization of HSV-2 infectivity, and other mechanism(s) seem to be involved in the protection.

**DISCUSSION**

In the present study, we demonstrated that the soluble form of the entire ectodomain of murine HVEM conferred remarkable antiviral effects on HSV-2 infection as compared with those of the entire ectodomains of human nectin-1 or human nectin-2 in mice. It was reported that activities of HSV-2 cell entry mediated by murine HVEM and nectin-1 were indistinguishable from those of human HVEM and nectin-1 [12]. However, human nectin-2 could serve as an entry receptor of HSV-2, but not murine nectin-2 [4]. Furthermore, murine HVEM, but not human HVEM, can bind to murine LIGHT, which is a natural ligand for HVEM and regulates T cell immune responses [19]. Therefore, we used murine HVEM, human nectin-1 and human nectin-2 for comparison of their antiviral potentials in transgenic mice. In transgenic mouse sera, expression levels of nectin-1Ig and nectin-2Ig were considerably higher than that of HVEMIg (Fig. 1b). However, the resistance to HSV-2 infection in the mice expressing HVEMIg was significantly higher than in the mice expressing nectin-1Ig and nectin-2Ig (Fig. 3). These findings suggest that murine HVEMIg is more effective than human nectin-1Ig and nectin-2Ig for protection against HSV-2 infection in transgenic mice.

The survival data in transgenic mice lines 2695, 2708 and 6473 were apparently correlated with the concentrations of nectin-1Ig in serum (Figs 1b and 3a). Additionally, sera of lines 2708 and 6473 could effectively neutralize HSV-2 infection *in vitro*. These results indicated that neutralization of HSV-2 is the main antiviral mechanism of nectin-1Ig in
transgenic mice. On the other hand, the transgenic mice expressing HVEMIg showed even more remarkable resistance against HSV-2 infections (Fig. 3), but their uninfected sera did not inhibit the viral infection in vitro (Table 1). These results indicate that neutralization with HVEMIg was not concerned with the antiviral mechanism observed in the mice expressing HVEMIg. Krummenacher et al. [20] reported that all clinical isolates of HSV-2 have the ability to use HVEM as a receptor despite the fact that it is expressed mainly on the immune systems which are not targets of productive HSV or latency in vivo. The principal natural function of HVEM is that it is known to regulate the T cell development signal (LIGHT signalling) through interaction with LIGHT, which is expressed in activated lymphocytes, natural killer cells and immature dendritic cells [21]. LIGHT signalling appears to be a strong potential candidate molecule to facilitate negative selection of T cells [21]. Wang et al., [22] reported that suppression of LIGHT signalling by HVEMIg inhibited negative selection, resulting in an increase in the percentage of CD4+CD8+ double-positive and CD8+ single-positive cells. In the transgenic mice, HVEMIg might compete the interaction of innate HVEM with LIGHT, with the result that strongly activated immune cells proliferated enough to overcome the HSV-2 infections. In fact, serological examination demonstrated that establishment of HSV-2 infection was confirmed in all of the surviving transgenic mice infected with HSV-2, but no symptoms were observed. Suppression of manifestation of the disease was also observed in the transgenic mice infected with HSV-1 (Fig. 2a, [10]). These observations may support the hypothesis that an activated immune response by HVEMIg is responsible for the resistance to HSV-2 infection in the transgenic mice expressing HVEMIg. To elucidate the mechanism of antiviral effects of HVEMIg, further investigation is needed, for example experimental infections with other pathogens in the transgenic mice.

HSV-2 infection in the vaginal epithelium required expression of either nectin-1 or HVEM, because HSV-2 could not infect nectin-1/HVEM double knockout mice [6]. Interestingly, the absence of nectin-1, but not HVEM, reduced efficiency of HSV-2 spread to the nerve system via both vaginal and intracranial routes of infection [6, 23]. These results indicated that nectin-1 plays an important role in systemic infections with HSV-2. It was reported that vaginal infection with HSV-2 was blocked by preincubation of the virus with soluble recombinant nectin-1 [24]. In the present study, a neutralization assay using the sera of transgenic mice line 2708 showed that over 90% of plaque formation of HSV-2 was inhibited (Table 1), demonstrating that nectin-1Ig has an effective antiviral potential. However, in the experimental infections using line 2708, only 50% of the transgenic mice survived against HSV-2 infection with 3 MLD50 (Fig. 3a), although the concentration of nectin-1Ig in sera was enough for viral neutralization. It has been reported that HSV-2 strains tend to cause more severe disease in animal models than HSV-1 strains [25, 26]. Therefore, this may be considered the reason for apparent resistance in the transgenic mouse line 2708 to HSV-2 infection with 3 MLD50 not being observed (Fig. 3a). In fact, we confirmed that almost all of the transgenic mice line 2708 survived until the end of the experimental period of infection with 3 MLD50 of HSV-1 (Fig. 2a). These results suggest that the antiviral effect of nectin-1Ig is more efficient against HSV-1 infection than HSV-2 infection in vivo.

Both the transgenic mouse lines 2216 and 2246 expressing nectin-2Ig did not show resistance against HSV-2 infection (Fig. 3a), whereas, in vitro, it has been reported that nectin-2Ig could exert the suppression effect of HSV-2 replication in transformed Vero cells [9]. There may be possible explanations for this discrepancy. In our previous study, concentration-dependent viral resistance of nectin-2Ig accumulating into the transformed cell lines was observed, suggesting that intracellular accumulation of nectin-2Ig over a definite amount is responsible for exertion of the antiviral effect [9]. However, the proliferation status of mucosal cells in the abdominal cavity of the transgenic mice was not synchronized because of the different life spans of various cell types. Therefore, it seems likely that accumulation of nectin-2Ig in all types of mucosal cells of the transgenic mice was not enough to prevent the viral replication, and consequently the mice were killed by the HSV-2 infection. In addition, we cannot exclude the possibility that the pancreatic exocrine defect observed in the transgenic mice expressing nectin-2Ig [13] may affect the protection against HSV-2 infection.

### Table 1. Inhibition of HSV-2 infection by soluble forms of gD receptors in sera of the transgenic mice

<table>
<thead>
<tr>
<th>Serum</th>
<th>Relative plaque number (% ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain 186</td>
</tr>
<tr>
<td>Uninfected mice</td>
<td></td>
</tr>
<tr>
<td>Wild-type*</td>
<td>100.0</td>
</tr>
<tr>
<td>Nectin-1Ig</td>
<td>4.5±4.5</td>
</tr>
<tr>
<td>Nectin-2Ig</td>
<td>6473</td>
</tr>
<tr>
<td>Nectin-2Ig</td>
<td>2216</td>
</tr>
<tr>
<td>HVEMIg A</td>
<td>111.2±7.9</td>
</tr>
<tr>
<td>HVEMIg C</td>
<td>111.2±9.3</td>
</tr>
<tr>
<td>Infected mice†</td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>10.7±4.2</td>
</tr>
<tr>
<td>HVEMIg A</td>
<td>4.8±6.1</td>
</tr>
<tr>
<td>HVEMIg C</td>
<td>8.1±5.7</td>
</tr>
</tbody>
</table>

*Uninfected non-Tg serum was used as a control of this assay.
†Mouse sera after 21 days post-infection with strain 186 at 300 MLD50 were used in this assay.
Genital HSV-2 infection causes significant morbidity [27] and is an important cofactor for HIV-1 infection [28–30]. Furthermore, in the past two decades, investigations have amply documented the increase in the frequency of genital HSV-1 compared with genital HSV-2 infection [31–33]. A vaginal microbiocide able to protect against HSV transmission could contribute significantly to controlling sexually transmitted diseases. New additional prevention and therapeutic strategies against both HSV-2 and HSV-1 infections are highly desirable. In the present study, we demonstrated the remarkable antiviral potential of HVEMIg in transgenic mice. Although drug efficacy studies in mouse models are not always translated to human trials, HVEMIg may be a candidate as a new medical agent against HSV infection and gene-based therapy in chronic HSV patients.

METHODS

**Viruses and cells**

HSV-2 strains 186 [34], YS-4 and 8620 KN [35] and HSV-1 strain VR3 [36] were propagated and subjected to titre determination using Vero cells grown in Dulbecco’s modified Eagle’s medium containing 5% foetal bovine serum.

**Generation of transgenic mice**

To construct a plasmid expressing nectin-1Ig that consists of the extracellular domain of human nectin-1 and the Fc portion of human IgG1, the chimeric gene fragment was inserted into the pCXN2 vector (pCXN2/nectin-1Ig) as described previously [18]. pCR4-TOPO/nectin-1 containing the complete human nectin-1 gene (GenBank accession number BC104948; Thermo Scientific) was used as a template for PCR. The PCR primer set used for the extracellular domain of human nectin-1 was as follows: 5¢-GGACCCCTCGAGGCCCCCGATGGCTCGGG-3¢ and 5¢-CTGAGCGGATCCGCGTGTTCGGGAGGAGAC-3¢. To generate transgenic mice expressing nectin-1Ig, the transgene prepared from the constructed plasmid was injected into C57BL/6 mouse eggs, and transgenic founders were identified by PCR with genomic DNA isolated from mouse tail [10]. PCR was performed using specific primers as follows: 5¢-GGACCCCTCGAGGCCCCCGATGGCTCGGG-3¢ and 5¢-CCGGATCTCTCGTGATCTG-3¢. Transgenic
mice expressing murine HVEMIg and human nectin-2Ig were previously generated [10, 13]. According to the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology in Japan, all mice were kept in a standard light/dark cycle with free access to food and water in the animal facility at our institute. Animal experiments were reviewed and approved by the Ethics Committee on Animal Experiments at Kyushu University.

Analysis of transgene expression

To confirm the expression of fusion proteins, human IgG-Fc affinity matrix (CaptureSelect, Life Technologies) was added to 20 µl mouse serum and incubated for 6 h at 4 °C. Subsequently, the beads were washed with PBS and used for Western blot analysis. Specific bands were detected by mouse anti-nectin-1 monoclonal antibody (1:2000, Santa Cruz Biotechnology) and alkaline phosphatase-conjugated goat anti-mouse IgG (1:3000, Sigma) for nectin-1Ig, rabbit anti-nectin-2 polyclonal antibody (1:3000, GeneTex) and alkaline phosphatase-conjugated goat anti-rabbit IgG (1:3000, Sigma) for nectin-2Ig, and goat anti-HVEM polyclonal antibody (1:3000, R&D Systems) and alkaline phosphatase-conjugated rabbit anti-goat IgG (1:3000, Sigma) for HVEMIg. All fusion proteins were also detected by Western blot analysis with alkaline phosphatase-conjugated goat anti-human IgG antibody, rabbit anti-hHVEM polyclonal antibody (1:3000, R&D Systems) and alkaline phosphatase-conjugated rabbit anti-goat IgG (1:3000, Sigma) for nectin-1Ig, rabbit anti-HVEM polyclonal antibody (1:3000, R&D Systems) and alkaline phosphatase-conjugated goat anti-human IgG antibody (1:3000, Sigma). To measure concentrations of the fusion proteins in sera of the transgenic mice, a competitive ELISA was performed as previously described [37].

Experimental infections in mice

Mice at 5–7 weeks of age were intraperitoneally inoculated with 3–300 LD₅₀ (10⁴–10⁶ p.f.u.) of HSV-2 strain 186 or 3 LD₅₀ of HSV-1 strain VR3 (10⁻⁵ p.f.u.). After inoculation, mice were observed twice a day for abnormal clinical signs such as loss of activity and tachypnea until post-infection day 21 (strain 186) or day 14 (strain VR3). The humane endpoints were severe weight loss and a failure to eat or drink. This experimental infection was also reviewed and approved by the Ethics Committee on Animal Experiments at Kyushu University.

Viral plaque reduction assay with soluble proteins

Inhibitory effects against HSV-2 infection of transgenic mice sera containing HVEMIg, nectin-1Ig or nectin-2Ig were examined on Vero cells seeded into 12-well plates. HSV-2 strains of 40 p.f.u. in 25 µl were incubated with 25 µl serum sample at 4 °C overnight, and each sample containing viruses was absorbed to Vero cells at 4 °C for 1 h. Viruses were removed and then overlaid with DMEM containing 0.5% SePlaque agarose (Lonza). Plaques were counted 3 days after infection.

Detection of antibody against recombinant gD of HSV-2 in mouse sera

Purified recombinant gD of HSV-2 were prepared as follows and used for an antigen of ELISA. Genomic DNA of HSV-2 strain 186 was extracted as described previously [9]. The cDNA of HSV-2 gD possessing the 6 x His-tag sequence at the C-terminus was amplified by PCR using the extracted genomic DNA and primer pair 5’-CCGGTCGACGACCA TGGGCGTTTGCCTC-3’ and 5’-CGAAGATCTCTTAC TAGTGGATGTTGATGATGTTAATTACGTG GTTCGACGAGG-3’. The amplified cDNA fragment was cloned into the pCAGGS expression vector [38]. The constructed plasmid was transfected into HEK-293 T cells, and after 48 h, cell lysates were collected as described previously [9]. Recombinant gD of HSV-2 contained in the cell lysate was purified by affinity chromatography with cComplete His-Tag Purification Resin (Roche). For detection of anti-HSV-2 gD antibody, ELISA was performed as described previously [9], using the purified recombinant gD, diluted sera from the transgenic mice as the primary antibody and alkaline phosphatase-conjugated goat anti-mouse IgG (1:5000, Sigma) as the secondary antibody.

Funding information

The authors received no specific grant from any funding agency.

Acknowledgements

We thank Dr. J. Miyazaki (Osaka University) for providing the pCXN2 vector, and Dr. Y. Yanagi (Kyushu University), Dr. H. Minagawa (Aichi Prefecture Institute of Public Health), Dr. F. Goshima (Nagoya University) for providing HSV-2 strains. We appreciate the technical support from the Research Support Center, Research Center for Human Disease Modeling, Graduate School of Medical Science, Kyushu University.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

7. Cocchi F, Lopez M, Menotti L, Aoubaïa M, Dubreuil P et al. The V domain of herpesvirus Ig-like receptor (HlRg) contains a major functional region in herpes simplex virus-1 entry into cells and...


13. Cocchi F, Menotti L, Dubreuil P, Lopez M, Campadelli-Fiume G. Cell-to-cell spread of wild-type herpes simplex virus type 1, but not of syncytial strains, is mediated by the immunoglobulin-like receptors that mediate virion entry, nectin1 (PRR1/HveC/HigR) and nectin2 (PRR2/HveB). J Virol 2000;74:3909–3917.


34. Sakuma S, Yamamoto M, Kumano Y, Mori R. An acyclovir-resistant strain of herpes simplex virus type 2 which is highly virulent for mice. Arch Virol 1988;101:169–182.

