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Development of a Small Animal Peripheral Challenge Model of Japanese Encephalitis Virus Using Interferon Deficient AG129 Mice and the SA14-14-2 Vaccine Virus Strain

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Abstract

Japanese encephalitis virus (JEV) is the most common cause of viral encephalitis in Asia, and it is increasingly a global public health concern due to its recent geographic expansion. While commercial vaccines are available and used in some endemic countries, JEV continues to be a public health problem, with 50,000 cases reported annually. Research with virulent JEV in mouse models to develop new methods of prevention and treatment is restricted to BSL-3 containment facilities, confining these studies to investigators with access to these facilities. We have developed an adult small animal peripheral challenge model using interferon-deficient AG129 mice and the JEV live-attenuated vaccine SA14-14-2, thus requiring only BSL-2 containment. A low dose of virus (10 PFU/0.1 ml) induced 100% morbidity in infected mice. Increased body temperatures measured by implantable temperature transponders correlated with an increase in infectious virus and viral RNA in serum, spleen and brain as well as an increase in pro-inflammatory markers measured by a 58-biomarker multi-analyte profile (MAP) constructed during the course of infection. In the future, the MAP measurements can be used as a baseline for comparison in order to better assess the inhibition of disease progression by other prophylactic and therapeutic agents. The use of the AG129/JEV SA14-14-2 animal model makes vaccine and therapeutic studies feasible for laboratories with limited biocontainment facilities.

Keywords

Flavivirus; Japanese encephalitis virus; interferon-deficient mice; AG129 mouse model; flavivirus pathogenesis; viral encephalitis; JEV SA14-14-2 vaccine strain

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Introduction

Japanese encephalitis virus (JEV) is a mosquito-borne virus from the genus *Flavivirus* in the family *Flaviviridae* historically found in southern and eastern Asia. It has become an increasing public health concern due to its recent geographic emergence into Pakistan, western Indonesia, Papua New Guinea and northern Australia [1,2]. JE is the most common viral encephalitis in Asia. According to the WHO, 50,000 cases of JE are reported annually, although this number may be inaccurate due to inadequate laboratory-based surveillance and reporting. Of the cases reported, 25–30% result in death, while 50% result in permanent neurologic sequelae. Most of the cases occur in children under the age of 15 in rural areas.

Several JEV vaccines are available, including inactivated whole virus formulations derived from infected mouse brains and cell cultures, and live attenuated virus preparations produced in primary hamster kidney and Vero cell cultures. No approved therapeutic drug is available for the treatment of JE. Vaccines from mouse brain tissue-derived inactivated virus manufactured in several Asian countries are expensive and require administration of several doses in a short time period to achieve adequate immunity [3]. The inactivated IXIARO vaccine, based on the attenuated vaccine strain SA14-14-2 grown in Vero cells, was licensed for use in 2009 in Europe, the United States and Australia. It provides antiviral immunity after 2 doses without adverse effects [4–7]. Even with available effective JEV vaccines, the virus continues to cause large disease outbreaks throughout Asia. In view of this, effective therapeutic agents, in addition to safe and affordable vaccines, are needed, and authentic animal models in which to test them are necessities.

Research with virulent JEV in mouse models to develop new methods for prevention and treatment is restricted to BSL-3 containment facilities. These animal models use neonate or weanling out-bred mice and intracerebral viral inoculation because susceptibility to wild-type (WT) JEV infection has been shown to be dependent on mouse age and route of inoculation [8]. These constraints on animal research greatly reduce the ability of researchers to evaluate JEV vaccine candidates and therapeutic agents.

We have previously developed interferon-deficient AG129 mouse models for assessment of prophylaxis and therapy of the viscerotropic flaviviruses. In this study, we have developed a new small animal model for an encephalitic flavivirus, using peripheral challenge of AG129 mice with live-attenuated JEV vaccine, SA14-14-2, which requires only BSL-2/ABSL-2 containment. We also have previously used these mice to develop BSL-2/ABSL-2 infection models for Venezuelan equine encephalitis (VEE) and West Nile (WN)/DEN chimeric virus infections [9–12]. The use of this animal model and intraperitoneal inoculation of virus makes JEV animal challenge studies a feasible option for laboratories with limited biocontainment. We present an in-depth characterization of the AG129/JEV SA14-14-2 model, detailing the viral disease progression, to provide measurements that in the future can be used as a baseline for studying vaccines and anti-viral therapeutic agents against JEV in this model.

Materials and Methods

JEV vaccine strain SA14-14-2 was originally obtained from the Arbovirus Diseases Branch, DVBD reference collection (Fort Collins, CO). Stock virus was grown in C6/36 cells to a titer of 2.25×10^8 PFU/ml. Vero cells were used in plaque assays and grown in MEM: DMEM supplemented with 10% FBS, 2mM l-glutamine, 0.15% sodium bicarbonate, 100 U/ml penicillin G sodium, and 100 µg/ml streptomycin at 37°C in 5% CO₂.

Animal Studies

AG129 mice deficient in interferon α/β and γ receptors were originally obtained from B&K Universal (Hull, United Kingdom) and bred in-house. Mice were handled as specified by the Division of Vector-Borne Diseases Institutional Animal Care and Use Committee recommendations (Protocol #12-026). JEV vaccine strain SA14-14-2 was evaluated for neuroinvasiveness in 6–8 week old AG129 mice. Nine groups of mice ($n=12$) were inoculated intraperitoneally (i.p.) with 10-fold dilutions of virus (10^6 to 10^{-3} PFU/0.1 ml). Before challenge, baseline body temperatures measured by implantable temperature transponders (BMDS) and weights were recorded daily for 3–4 days. After challenge mice were monitored 4 times per day for an increase followed by a decrease in normal temperature, weight loss and other signs of morbidity (hunched posture, neurological signs). Mice that showed signs of morbidity were euthanized immediately.

To determine growth rate of JEV SA14-14-2 in tissues, 6 week-old AG129 mice ($n=5$) were infected with 10 PFU/0.1 ml i.p., and mice were euthanized for sample collection at 6 time points (24, 48, 66, 78, 90 and 102 hpi). Blood was collected via cardiac puncture and placed immediately into microtainer tubes for serum collection. Brain and spleen were harvested post-mortem, rinsed with PBS, manually chopped into sections, and placed in pre-weighed MagNA Lyser Green Bead tubes (Roche Diagnostics) containing either 500 μ l of BA1 for plaque assay, 500 μ l RNA Later (Qiagen) for viral RNA quantitation or 500 μ l TE buffer (2mM EDTA, 50 mM Tris-HCl, pH 7.4) containing protease inhibitors [1 mg/ml of Pefabloc SC and 1 μ g/ml each of aprotinin, antipain, leupeptin and pepstatin (Roche)] for rodent multi-analyte profile (MAP) analysis (Myriad RBM). Tissue samples were weighed and homogenized for two 30s cycles at 4000 rpm using a Roche MagNA Lyser (Roche Diagnostics). Homogenized samples were centrifuged for 5 minutes at 10,000 rpm at room temperature and stored at -80°C along with serum samples prior to analysis.

Statistical analysis of body temperatures in infected mice

A semiparametric, mixed model was fit to the longitudinal temperature data of the mice. The model includes treatment-specific temperature curves, a component to model the diurnal pattern of body temperature, and mouse-specific curves. Each of the components was characterized by a second degree penalized spline with a truncated power basis. The solution to the fit and estimated variances were obtained by computing the best, linear, unbiased predictors of the penalized spline's representation as a linear, mixed model [13].

Plaque assay

Viral plaque titrations of serum and tissue samples from 6 time points were performed under double agarose overlay in six-well plates of confluent Vero cells [14]. Serum and tissue samples homogenized in BA-1 diluent were serially diluted in the same medium. Each dilution (100 μ l) was added to a confluent monolayer of cells and incubated at 37°C for one h with gentle rocking. Cells were overlaid with 3 ml of LE agarose in YE-LAH medium as described previously [14]. Plates were incubated for 4 days before 3 ml of a second overlay containing 0.008% neutral red in the same medium was added to each well. Plaques were counted 24 and 48 h later.

RNA extraction from tissues and sera and *in vitro*-transcribed RNA controls

Viral RNA from 140 μ l of serum samples was extracted from virus supernatant with a QIAmp Viral RNA kit (Qiagen), and total RNA was purified from 100 μ l of tissue homogenate using the RNeasy Mini kit (Qiagen) following the manufacturer's protocols. The JEV consensus primers and probe for quantitative RT-PCR were developed and kindly provided by Dr. Barbara W. Johnson, Diagnostic and Reference Lab, Arbovirus Diseases

Branch, Division of Vector-Borne Diseases, CDC (Supplemental Table 1). To prepare *in vitro* transcribed RNA from the 5' untranslated region (UTR) of the genome for a copy number control, the JEV consensus primers (JEVF and JEVr) with the T7 promoter sequence (TAATACGACTCACTATAGGGAGA) added to the 5' end of JEVF primer were used to amplify a 105 bp segment of cDNA. The same size segment of RNA was transcribed from the cDNA using the mMessage mMachine kit (Life Technologies) according to the manufacturer's protocol. RNA was quantified using the RNA Nano kit in the Agilent Bioanalyzer, and RNA copy numbers/ml were calculated based on spectrophotometry readings.

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

A 5 µl aliquot of each purified RNA sample was added to master mix from iScript One-step RT-PCR kit (Bio-Rad) containing primers and probe described above: 25 pmol of probe JEVp, 100 pmol of forward primer JEVF and 100 pmol of reverse primer JEVr (Supplemental Table 1). JEV-specific RNA extracted from serum and tissue samples was analyzed in triplicate by qRT-PCR using the homologous RNA standards on a Bio-Rad IQ5 Real-time PCR detection system under the following conditions: 50°C for 30 min, 95°C for 15 min, followed by 45 cycles of 94°C for 15 sec, and 55°C for 30 sec with continuous fluorescence data collection.

Rodent multi-analyte profile (MAP)

Serum and tissue samples homogenized in TE buffer containing protease inhibitors from each time point were pooled ($n = 5$ samples/pool) and frozen at -80°C . Pooled samples were shipped to Myriad RBM for analysis of 58 biomarkers on the RodentMAP[®] version 3.0 platform which uses microspheres infused with fluorescent dyes and coated with reagents that bind target analytes in serum or tissue homogenates. The least detectable dose (LDD) is defined as 3 standard deviations above mean background measured for each analyte in each multiplex.

Results

Determination of effective challenge dose of JEV SA14-14-2 in AG129 mice

Previously, we determined that AG129 mice lacking an intact IFN response were susceptible to virulent DENVs and WNV, and VEEV and YFV vaccine strains. In order to determine if these mice were susceptible to JEV vaccine strain SA14-14-2, mice were challenged via i.p. inoculation with varying doses of virus ranging from 10^6 to 10 PFU/0.1 ml. The onset of morbidity occurred in a dose-dependent manner with mice receiving 10^6 PFU/0.1 ml of virus showing outward signs of morbidity earliest. Sick individuals, as indicated by an increase followed by rapid decrease in baseline body temperatures and neurological signs, were euthanized as early as 66 hpi (data not shown). Mice receiving 10 PFU/0.1 ml of JEV SA14-14-2 began to show outward signs of morbidity at 96 hpi (Fig. 1). The mean survival time (MST) of mice challenged peripherally with JEV SA14-14-2 was shown to be dose-dependent with shortened MSTs correlating with higher-titer inocula. Mice inoculated with 10 PFU/0.1 ml of virus had a MST of 106 h ($\text{SD} \pm 7.9$). Mice inoculated with 1 or 0.1 PFU/0.1 ml of virus had MSTs of 130 h ($P = 0.016$) and 122 h ($P = 0.094$), respectively (Table 1). At 144 hpi mice challenged with 1 or 0.1 PFU/0.1 ml of virus had survival rates of 58% and 83%, respectively (Fig. 1).

Determination of clinical sign onset with effective challenge dose (10 MD_{50})

In order to determine an accurate measurement of the time of onset of clinical signs, baseline body temperatures were measured by implantable temperature transponders for 3–4

days prior to infection. After challenge, body temperatures of infected mice were monitored 4 times per day in addition to weight change and neurological signs of disease. Body temperatures of infected mice rose observably between 72 and 96 hpi (Fig. 2A and 2B). Statistical analysis suggests this increase in the mean temperature of infected mice begins by 57 hpi as indicated by the upper bound of a 95% confidence for the mean temperature curve of infected mice (Fig. 2C).

Determination of viral load in tissues from JEV SA14-14-2-infected AG129 mice

An analysis of viral replication in the sera, spleens and brains of infected animals was performed by viral plaque assay and qRT-PCR. Infectious virus and viral RNA were first detected in sera of mice infected with 10 PFU/0.1 ml of virus at 24 hpi by plaque assay and qRT-PCR with average titers of 1.6 log₁₀ PFU/ml and 3.0 log₁₀ RNA copies/ml, respectively. Virus and RNA titers peaked in sera at 102 hpi with average titers of 9.9 log₁₀ PFU/ml and 10.9 log₁₀ RNA copies/ml (Fig. 3A and 4A). Infectious virus in the spleen was first detected at 48 hpi by plaque assay with an average titer of 5.8 log₁₀ PFU/g; however, viral RNA could be detected at 24 hpi with an average titer of 4.3 log₁₀ RNA copies/g. Infectious virus and viral RNA titers peaked in the spleen at 102 hpi with average titers of 10.5 log₁₀ PFU/g and 11.9 log₁₀ RNA copies/g, respectively (Fig. 3B and 4B). Infectious virus in the brain was also detected by plaque assay at 48 hpi with an average titer of 3.7 log₁₀ PFU/g, while viral RNA was detectable in the brain at 24 hpi with an average titer of 4.0 log₁₀ RNA copies/g. Infectious virus and viral RNA rose steadily in the brain and reached highest titers at the last time point with average titers of 8.1 log₁₀ PFU/g of infectious virus and 8.6 log₁₀ RNA copies/g of viral RNA (Fig. 3C and 4C). These findings of infectious virus titer and presence of viral RNA in the tissues correspond to morbidity onset of animals determined by increased body temperature, calculated to peak at 57 hpi.

Analyte biomarker profile to assess inflammation or tissue damage as a result of JEV SA14-14-2 infection in AG129 mice

A 58 analyte pro- and anti-inflammatory and tissue damage biomarker profile was constructed with pooled samples of serum and tissue homogenate supernatants from brain and spleen of JEV SA14-14-2-infected AG129 mice throughout the course of infection. Overall, spleen tissue homogenates showed 4-fold or greater increases in concentration in the largest numbers of analytes (47 of 58) compared to mock-infected animals. Analyte concentrations that increased in the spleen tended to peak at 78 hpi, decrease slightly by 90 hpi and increase again by 102 hpi. Overall analyte concentrations in serum (26 of 58) and brain tissue homogenates (16 of 58) also increased when compared to samples taken from mock-infected animals. Analyte concentrations in the brain and serum that were shown to increase peaked at 90 hpi and remained high until the last time point at 102 hpi. Particular analytes known to increase in JEV infection in humans that were also shown to have a marked increase in SA14-14-2-infected AG129 mice included IL-6, macrophage inflammatory protein (MIP)-1 α , MIP-1 β , monocyte chemotactic protein (MCP)-1, T-cell specific protein RANTES (RANTES), tumor necrosis factor (TNF) α , and matrix metalloproteinase (MMP)-9.

Table 2 summarizes those analytes from sera and tissues of AG129 mice that exhibited a 4-fold or greater change in concentrations as a result of JEV SA14-14-2 infection compared to mock-infected animals. Only those analytes associated with inflammation or tissue damage are included. A more detailed report of the concentrations of each analyte in the different tissue samples at each time point is presented in Supplemental Table 2.

Discussion

JEV continues to be a public health concern as the most common cause of viral encephalitis in Asia and due to its continued geographic expansion into Pakistan, western Indonesia, Papua New Guinea and Australia [1,2,15]. New vaccines and treatments are being developed to combat the spread of the virus, and appropriate small animal models are needed in order to test these anti-viral agents. In this study, we report the use of the AG129 interferon-deficient mouse as an animal model for JEV using the vaccine strain SA14-14-2. We have previously shown these mice to be susceptible to both viscerotropic and encephalitic flaviviruses and alphaviruses including DENV, YFV, WNV and VEEV [9–12]. A low dose of JEV SA14-14-2 (10 PFU/0.1 ml) induced 100% morbidity in infected mice with a mean survival time of 106 hpi (± 7.9). Increased body temperatures of infected mice beginning at a calculated time point of 57 hpi correlated with a marked increase in titers of infectious virus and viral RNA in the serum and tissues. With the RodentMAP[®] version 3.0 platform a 58-analyte profile was developed to determine baseline concentrations of important biomarkers during infection. The MAP showed a marked increase in pro-inflammatory cytokines in serum and tissue samples, especially in the spleen.

Vaccines developed for prophylaxis of JEV infection originally were made from infected mouse brain tissue or primary hamster kidney cells [16,17]. The JEV vaccine strain SA14-14-2 was developed in China and has been used for prophylaxis in that country since 1988 [16,18–21]. Recently, a vaccine consisting of inactivated JEV SA14-14-2 grown in Vero cells was developed and licensed in the U.S., Europe, Japan and Australia [4–6,22,23]. The mechanism of attenuation of JEV SA14-14-2 is attributed to several amino acid (AA) substitutions in both the E protein and the nonstructural proteins and nucleotide substitutions in UTRs of the genome [24–29]. Overall there are 29 amino acid differences between WT SA14 and vaccine strain SA14-14-2 [25]. The mutation in the E protein at AA 138 from a glutamic acid (E) to a lysine (K) results in an increased dependence on glycosaminoglycans like heparin for virus-cell attachment and entry [28,29]. This mutation was also shown to increase viral sensitivity to IFN α by preventing the virus from blocking IFN signaling [27]. The type I IFN response restricts flaviviral spread and dissemination to the CNS [30,31]. JEV SA14-14-2 may lack neurovirulence due to inducing the maturation of dendritic cells that activate anti-viral cytokines such as IFN and TNF α [18,32]. JEV SA14-14-2 infection activates dendritic cells and other monocytes while WT virus suppresses their maturation and function [32–34]. Due to its increased susceptibility to IFN in the mouse model we wanted to determine if this virus could cause morbidity in interferon-deficient AG129 mice. In this study JEV SA14-14-2 produced 100% morbidity in 6–8 week old AG129 mice with doses as low as 10 PFU per mouse. The lack of an effective IFN response in AG129 mice is most likely the reason this highly attenuated virus with increased sensitivity to IFN was able to infect and disseminate to the CNS in infected mice in this study.

Since AG129 mice are highly sensitive to viral infection, they are an excellent animal model to study other live-attenuated vaccine candidates developed for flaviviruses, and any potential vaccine that does not cause disease in these animals may be considered a very safe candidate due to the high sensitivity of AG129 mice to viral infection [10,11]. In fact, a WNV/DENV2 chimera vaccine featuring the structural proteins of WNV in the DENV2 backbone was shown to be completely attenuated in this animal model while still producing an active infection that resulted in production of neutralizing antibody to WNV [11]. This animal model has also been shown to be valuable for initial testing of potential therapeutics to flaviviruses. We found that the humanized MAb 2C9-cIgG was able to protect AG129 mice from lethal YFV-17D challenge at a dose of 1.27 μ g/mouse when administered 24 hours prior to infection [35].

Rodent models to study pathogenesis of JEV are available but require neonate or weanling animals in BSL-3 facilities. Age and route of inoculation are key factors for susceptibility to flaviviruses in mice [8]. Up to 3 to 4 weeks of age, immunocompetent mice are susceptible to a low dose peripheral challenge with flaviviruses, but after this time signs of viremia and infection are not apparent [28]. This may be because JEV has a greater affinity for and replicates more efficiently in immature neurons [36]. Other rodent models for JEV challenge have included $\beta_2m^{-/-}$, C57BL/6, C3H/HeN, BALB/c, ICR, Swiss mice and suckling hamsters by intracerebral or peripheral challenge [37–41]. Interestingly, the β_2 microglobulin-deficient $\beta_2m^{-/-}$ mice are deficient in CD8⁺T cells and are more susceptible to viral infection due to a reduced IFN γ response [42]. The AG129/JEV SA14-14-2 model of infection developed in this study is a safer and more broadly applicable model since it allows for studies to be conducted under reduced biocontainment (BSL-2/ABSL-2) in adult mice with i.p. inoculation that more closely resembles the route of natural infection compared to other animal models with WT virus inoculated intracerebrally.

Using the RodentMAP[®] version 3.0 platform we constructed a comprehensive pro-inflammatory biomarker profile of JEV SA14-14-2 infection of AG129 mice. We previously used this technology to construct a similar profile of the AG129/YF17D-204 infection model. During infection with YF17D-204, the greatest fluctuation in analytes over the course of infection occurred in the brain. Significant increases in pro-inflammatory analytes in the serum and liver were detected in a bi-phasic pattern [12]. In contrast, we found a sustained increase in pro-inflammatory analytes, particularly in the spleen, in the AG129/SA14-14-2 model.

Analytes identified as important in human JEV infections include TNF α , IL-6, IL-8 and RANTES [43,44]. Winter *et al.* [43] correlated an increase of IL-6, IL-8, and TNF α in CSF along with an increase in RANTES in the plasma of JEV infected patients with a negative disease outcome. In this study we saw similar results with an increase in IL-6 and RANTES in the serum, brain and spleen, along with an increase in TNF α in the spleen. TNF α is a pro-inflammatory cytokine produced mainly by activated macrophages and to some extent CD4⁺T cells, natural killer (NK) cells and neurons. It is important in stimulating the acute phase reaction to infection along with IL-1, IL-6 and IL-8. An increase in TNF α through TLR-3 signaling can lead to decreased integrity of the blood-brain barrier, allowing virus particles and virus-infected immune cells access to the CNS [45,46]. Exposure to pro-inflammatory cytokines over long periods of time in combination with low levels of anti-inflammatory cytokines such as IL-10 and IL-4 is linked to more severe cases of encephalitis [47]. Interestingly, after vaccination of humans with JEV SA14-14-2 live attenuated vaccine, levels of IL-8 in the serum along with MCP-1, MIP-1 α and MIP-1 β and IL-6 to a lesser extent were significantly higher than those not receiving the vaccine, and TNF α and IFN γ were minimally detectable in the vaccinated population [48]. In the AG129/SA14-14-2 model we also found an increase in MCP-1 in serum, brain and spleen and an increase in MIP-1 α in the spleen and MIP-1 β in serum and spleen. The strong increase of pro-inflammatory cytokines, especially in the spleen, after infection with JEV SA14-14-2 combined with the inability of AG129 mice to utilize an IFN response against viral infection appear to be the main reason for the attenuated virus's ability to cause disease in this mouse model.

The AG129/SA14-14-2 animal model results in a lethal encephalitic infection that mimics the progression of disease from virus infection in the peripheral tissues to the CNS and the upregulation of pro-inflammatory analytes associated with fatal human infections. Since this model uses the JEV vaccine strain, research is not limited to high biocontainment facilities, allowing for the evaluation of vaccines and therapeutics to JEV under reduced containment. Compared to models utilizing virulent JEV strains, the AG129/SA14-14-2 animal model is a

safe and effective tool to investigate the pathogenesis of JE viral disease and evaluate future vaccine candidates and therapeutic agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- An animal model using mice that lack interferon and a vaccine strain of JEV was developed.
- A low dose of virus was able to induce 100% morbidity in infected mice.
- Fever in mice correlated with the dissemination of infectious virus in serum and tissues.
- Increased pro-inflammatory analytes in serum and tissues correlated with disease progression.
- The model allows for the ability to effectively evaluate JEV vaccine and therapeutic candidates.

Figure 1. Dose-dependent virulence of JEV SA14-14-2 in AG129 mice
Mice ($n=12$) were inoculated i.p. with 10 (●), 1 (□) and 0.1 (○) PFU of JEV SA14-14-2.

Figure 2. Body temperatures of uninfected and JEV SA14-14-2 infected AG12 mice
 Panel A: Mean of observable body temperatures of infected (solid line) and uninfected (dashed line) mice. Panel B: Estimated mean body temperatures from model with mouse-specific and diurnal effects removed for infected (solid) and uninfected (dashed) mice. Panel C: Difference in estimated mean body temperatures (infected minus uninfected) with 95% confidence bound (dashed lines).

Figure 3. Infection virus titers in various tissues of AG129 mice infected with JEV SA14-14-2 expressed as PFU/gram (or ml)

Panels: A, serum; B, spleen; C, brain. Plaque assays of tissue samples taken at 6 different time points during infection were performed in duplicate.

Figure 4. Virus RNA in various tissues of AG129 mice infected with JEV SA14-14-2 expressed as genomic copies/gram (or ml)

Panels: A, serum; B, spleen; C, brain. Virus RNA titers of tissue samples taken at 6 different time points during infection were measured by quantitative RT-PCR. Each assay was performed in triplicate.

Table 1

Mean survival times (MST) of AG129 mice infected with JEV SA14-14-2.

Challenge dose (PFU/0.1ml)	% morbidity (survivors/total)	MST (hours) $\pm$ SD
10	100 (0/12)	106 $\pm$ 7.9
1	42 (7/12)	130 $\pm$ 17.1
0.1	17 (10/12)	122 $\pm$ 8.5

Table 2

Analytes associated with inflammation or tissue damage with a four-fold or greater change in concentration as a result of JEV SA14-14-2 infection in AG129 mice.

		Serum	Brain	Spleen
Pro-inflammatory				
CRP	C-Reactive Protein Mouse	↑	↑	↑
CD40	CD40			↑
CD40L	CD40 Ligand	↑		
	Fibrinogen		↑	↑
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor			↑
KC/GRO	Growth-Regulated Alpha Protein	↑	↑	↑
IFN γ	Interferon gamma	↑		↑
IP-10	Interferon gamma induced protein	↑	↑	↑
IL-1 α	Interleukin-1 alpha	↑		↑
IL-1 β	Interleukin-1 beta			↑
IL-6	Interleukin-6	↑	↑	↑
IL-11	Interleukin-11			↑
IL-12p70	Interleukin-12 subunit p70	↑		
IL-18	Interleukin 18			↑
MDC	Macrophage-derived chemokine	↑	↑	↑
MIP-1 α	Macrophage Inflammatory protein-1 alpha			↑
MIP-1 β	Macrophage Inflammatory protein-1 beta	↑		↑
MIP-1 γ	Macrophage Inflammatory protein-1 gamma		↑	↑
MIP-2	Macrophage inflammatory protein-2	↑	↑	↑
MIP-3 β	Macrophage inflammatory protein 3 beta			↑
MCP-1	Monocyte chemotactic protein-1	↑	↑	↑
MCP-3	Monocyte chemotactic protein-3	↑	↑	↑
MCP-5	Monocyte chemotactic protein-5	↑	↑	↑
OSM	Oncostatin-M			↑
RANTES	T-cell Specific Protein RANTES	↑	↑	↑
TNF α	Tumor Necrosis Factor alpha			↑
Anti-inflammatory				
IL-10	Interleukin-10	↑		↑
TMP-1	Tissue Inhibitor of Metalloproteinases	↑	↑	↑
Tissue damage				
IL-7	Interleukin-7	↑		↑
MMP-9	Matrix Metalloproteinase-9			↑
MPO	Myeloperoxidase	↑	↑	↑
	Myoglobin	↓		

		Serum	Brain	Spleen
PAI-1	Plasminogen Activator Inhibitor-1	↑	↑	↑