Developing a new immunocompetent mouse model for Dengue virus infection

Mirian Mendoza, Mohanraj Manangeeswaran, Derek D.C. Ireland, Daniela Verthelyi
Laboratory of Immunology, Center of Excellence in Infectious Disease and Inflammation, Office of Biotechnology Products, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD, United States

Abstract

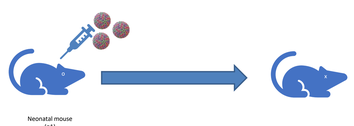
Antibodies play a key role in blocking viruses from infecting cells and increasing their clearance. However, some viruses, like Dengue, can infect cells more readily when bound by antibodies of sub-optimal specificity or levels, resulting in more severe infections, this is called antibody dependent enhancement. Currently there are no immune competent animal models to examine whether therapeutic antibodies could elicit ADE. Dengue is the virus infection where ADE is more clearly observed, therefore, we developed the first immune competent mouse model of Dengue. This study shows that immunocompetent neonate C56BL/6 mice challenged subcutaneously with DENV develop viremia followed by high levels of virus in the CNS and eyes starting at 6 days post infection. The mice exhibit clear signs of neurological disease such as unsteady gait, ataxia, and tremors and succumb to infection 9-12 days after challenge. The infection in the CNS is associated with significant increase in mRNA expression for interferon-inducible genes, chemokines, antigen presentation and activation, complement as well as apoptosis which correlate with the level of infection. Moreover, immunohistochemistry and flow cytometry demonstrate an increase in infiltrating immune cells in the CNS of DENV infected mice. Currently we are using this model to study the impact of immune modulators alone or in combination with mAbs and small molecules on disease progression and survival to establish the model as a tool to test the impact of different product quality attributes on anti-DENV therapeutic safety and efficacy.

Background

Animal models can help us understand the pathophysiology of the virus, identify therapeutic targets, and explore the safety and efficacy of new therapeutics and vaccines. Dengue is a mosquito-borne disease that is endemic in most tropical countries. It continues to be a public health concern as it infects about 390 million people each year worldwide. Currently, there is no effective antiviral therapy or licensed vaccine available against Dengue. Therefore, disease monitoring and prevention is currently limited to vector control measures. Animal mouses models of Dengue infection allow us to not only study the viral pathogenesis of dengue virus but most importantly test potential therapeutics and vaccines. However, murine models have been a particular challenge for DENV considering that current traditional mouse models of Dengue infection rely on mice deficient in or fully lacking interferon (IFN) or STAT receptors elements whose signaling are important to pathogenesis in humans and vaccine responses. Our aim was to develop an immunocompetent mouse model for Dengue virus infection to study viral pathogenesis and also test therapeutics.

Materials and Methods

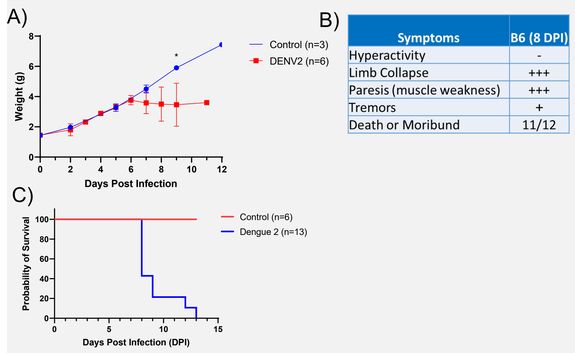
DENV2 Neonatal Mouse Model



P1 Black 6 WT mice are infected with DENV2 (NGC strain; 3600TCID50) subcutaneously.

Results and Discussion

DENV Model



Symptoms	B6 (8 DPI)
Hyperactivity	-
Limb Collapse	+++
Paresis (muscle weakness)	+++
Tremors	+
Death or Moribund	11/12

Figure 1: Weight loss (A), symptoms of disease (B) and mortality (C) of WT neonatal B6 mice infected with DENV2. B6 wildtype mice were subcutaneously infected with DENV2 24 hours after birth and monitored for weight loss, signs of disease, and mortality over time. DENV2-infected wildtype neonates drop to 100% mortality within 15 days post infection.

DENV2 primarily infects neurons in the CNS of newborn B6 mice

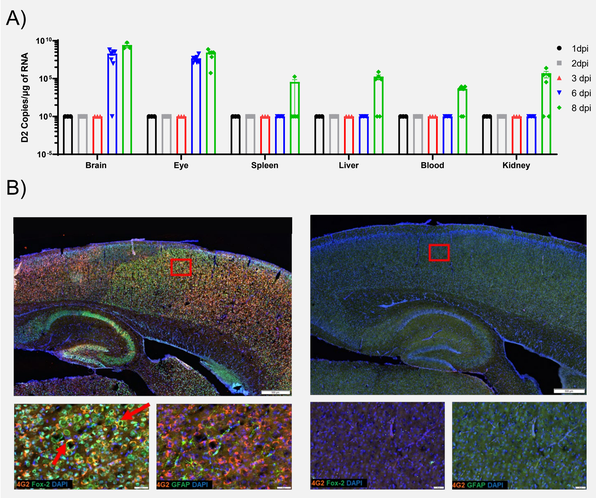


Figure 2. Viral load of DENV2 in B6 WT neonatal mice infected with DENV2
(A) DENV2 RNA was quantified in the CNS and peripheral organs of infected B6 WT at 1dpi, 2dpi, 3 dpi, 6 dpi and 8 dpi (N=3-6 per group) using quantitative real-time PCR. Values are presented as the number of viral RNA copies/mg of RNA. (B) Virus infected cells in the brain of B6 WT. IF-IHC staining of brain of B6 WT uninfected (right) and infected (left) with DENV2 (8 dpi). DENV2 virus labeled with 4G2 ab (orange), astrocytes (GFAP) or neurons (Fox-2) in green, and nuclei with (DAPI) in blue. Scale bar: 20mm. Note that 4G2 (DENV) colocalizes with Fox-2 (neurons) but not GFAP (astrocytes).

Inflammatory response in the CNS of DENV infected B6 mice

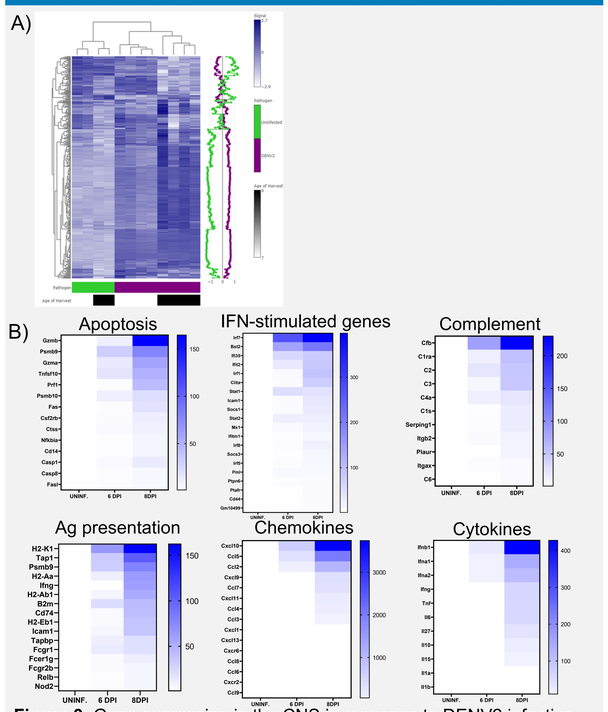


Figure 3. Gene expression in the CNS in response to DENV2 infection. (A) Nanostring mouse immunology panel. Heatmap shows gene expression (normalized counts) in the brain DENV2 infected (purple) at 6 dpi (white) and 8 dpi (black) and uninfected age-matched control (green). Each column represents an individual mouse (n = 2-4/group) and the green and purple lines on the right depict the average normalized gene expression. (B) Heatmaps depict the geometric mean change in gene expression in brain tissue of DENV2 infected mice relative to age-matched uninfected mice.

Macrophages and NK cells infiltrate the CNS

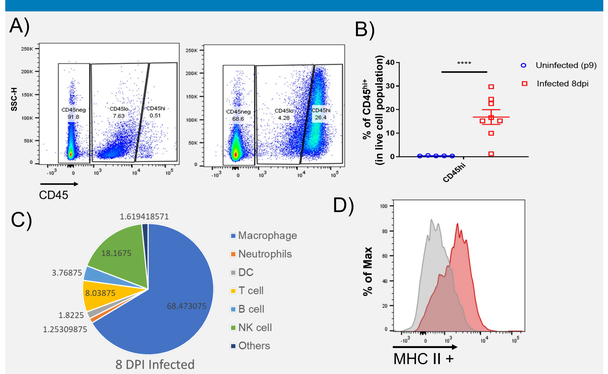


Figure 4. Immune cells infiltrating the CNS in response to DENV2 infection. Flow cytometry performed on cells isolated from the CNS (n=8) of DENV2 infected B6 WT at 8 dpi. (A) Live cells were gated based on CD45 expression. (B) Percent of CD45^{hi} cells in total live cells. (C) Phenotype of CD45^{hi} infiltrating cells in CNS. (D) MHCII expression in CD45^{low} microglial cells from the CNS of mice infected with DENV2 (red) compared to uninfected age-matched control (gray).

CPG ODN, but not ST-148, improve survival rate of DENV2 infected mice

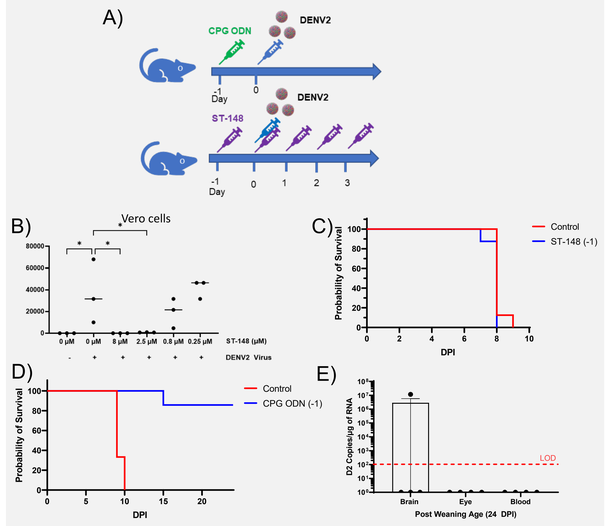


Figure 5. CPG ODN but not ST-148 improves the survival rate of DENV-infected mice. (A) Schematic of experimental design. Top: P0 WT B6 mice were treated with CPG ODN (50ug) subcutaneously and after 24 h infected with a lethal dose of DENV2. Bottom: P0 WT B6 mice were treated with ST-148 (50mg/kg) 24 h before challenge with DENV2 and received for four additional doses of ST-148. (B) ST-148 reduces viral load in Vero cells challenged with DENV2. VERO cells were pretreated with ST-148 2hrs before challenge with DENV2 virus (MOI 0.1). (C) Survival curve of mice treated with ST-148. (D) Survival curve of mice treated with CPG ODN (50ug). Survival rate was reduced I animals treated at the time of infection. (E) 24 days post infection, DENV2 RNA levels in the CNS and peripheral organs of infected B6 WT mice that received CPG ODN treatment and survived.

Conclusion

- Neonate B6 mice are susceptible to DENV infection.
- DENV2 infection is lethal in B6 neonate mice.
- DENV2 can be detected in the blood, spleen and kidney but primarily infects neurons in the brain of B6 mice.
- Gene expression and pathway analysis showed that DENV2 infection in the CNS of infected mice induces a strong upregulation of genes linked to complement and apoptosis activation, innate immune response, as well as interferon signaling. More specifically, there was a strong upregulation of *Irf-7*, *Bst-2*, *Ifi35* indicating a strong type I interferon response. The elevated levels of chemokines such as *CXCL10* suggest an upregulation in cellular infiltration, while the increased mRNA levels of cytokines *Tnf* and *Ifn* are indicative of robust inflammation.
- Flow analysis showed that most of the cells infiltrating the CNS in response to DENV2 infection are macrophages and NK cells consistent with the gene expression analysis showing an increase in inflammation and innate immune response.
- The *in vivo* model allows for testing of potential therapeutics. Initial studies show that CPG ODN improves survival but ST-148 does not.

Acknowledgements

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