Development of an immunocompetent mouse model of Dengue virus infection that can be used to test the efficacy of therapeutics and understand Antibody Dependent Enhancement of disease.

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Abstract

Dengue virus (DENV) infects over 400 million people worldwide every year. While most 1st infections are mild, second infections with a heterologous DENV can result in severe disease, encephalitis and death through antibody dependent enhancement (ADE). There are no approved effective therapeutics and no licensed vaccines for dengue virus disease. There are no immunocompetent mouse models available to understand host-pathogen interactions, establish determinants of disease, or explore risk factors for ADE. To address this unmet need, we developed an immunocompetent neonatal mouse model in C57BL/6 mice using DENV serotype 2, New Guinea C strain (DENV2). The DENV2 infected mice fail to thrive, and develop lethargy, ataxia, and tremors around 6- 9 dpi and approximately 50% of the infected mice succumbed to disease by 10-12 dpi. The mice have a viremia followed by high levels of virus in the CNS and eyes starting 3 days post infection (DPI). The infection in brains and eyes is associated with significant increase in mRNA expression for interferon-inducible genes (BST2, STAT1&2, IRGM1, IFIT2, IRF7, IFI35), RIG-I-Like receptors (DDX58 and IFIH1), chemokines (CXCL10, CCL5, CCL12, CCL2) antigen presentation and activation (H2-K1, CTSS, TAP1, TAPBP, PTPRC) complement (C4A, C1QB, C2, C1QA) and FC receptors (FCGR1, FCGR4, FCGR2B) as well as a corresponding reduction in genes related to neuronal function (PCP2, PAX2). This model will be used to test the efficacy of anti-DENV therapeutics and understand the determinants of ADE using heterologous infections and antibody transfer studies.

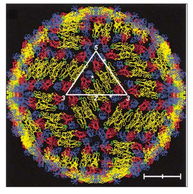
Introduction

Biology of Dengue Virus

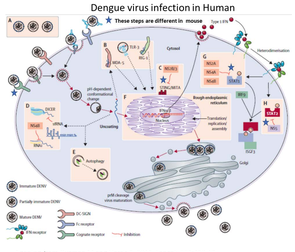
Family: Flaviviridae
Genus: Flavivirus
Species : Dengue

Dengue virus is presently the most common cause of arboviral disease globally. All four serotypes can be found worldwide. WHO estimates an annual incidence of ~ 100 million infections, with approximately 500,000 people with Dengue Hemorrhagic fever (DHF).

Enveloped virus with single positive sense RNA ~ 10 kb. Four serologically and genetically distinct serotypes Dengue 1-4.

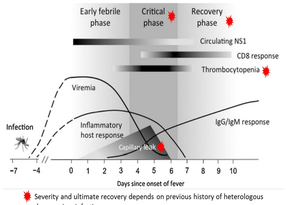


Life Cycle of Dengue viruses



Adapted from Guzman M and Harris E, 2015, Lancet, 385:453-65

Clinical phases in the evolution of Dengue



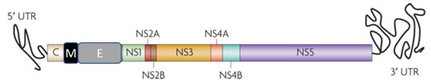
Adapted from Simmona CP et al., Vaccine 33 (2015) 7061-7068

Materials and Methods

THE SYSTEM

Mice: Neonatal B6-wt
Virus: Dengue virus serotype 2 (New Guinea C)
Route of infection: Sub-Q (scapular)
Dose: 2x10^3 PFU

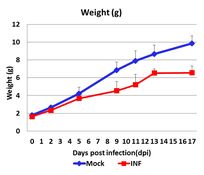
DENV2 Molecular clone used in the study

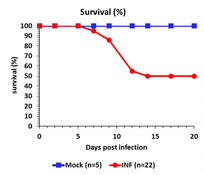


Plaque morphology of DENV2 molecular clone

Results and Discussion

DENV2 infection is lethal in neonatal mice





Dengue Clinical Score at 9 dpi

Symptoms	Score Range	Average score
Tremors	1-3	1.3
Hyperactivity	1-3	0.3
Lethargy	1-3	2
Hunched posture	1-2	0.3
Gait -Ataxic (wide, drunk stance)	1-2	2
Gait- Neuropathic (Leg drag, weak dorsiflexion)	1-2	0.3
Hind Limb paralysis	1-2	0
Moribund	1	0

Fig. 1. DENV2 causes disease in neonatal C57B6 mice. A) B6-wt mice infected sub-cutaneously with 2x10^3 PFU DENV2 at p1 gained weight normally for 5 days post infection but the rate of weight gain was significantly lower compared to uninfected controls and **B)** infected mice showed higher rate of mortality. Infected mice show symptoms and succumb to disease by 8-12 dpi. **C)** Mice are scored based on symptoms and average score of infected mice at 9 dpi are presented.

DENV2 peripheral infection reaches the CNS and eye

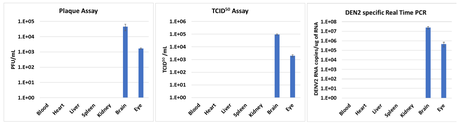


Fig. 2. Peripheral **DENV2 infection reaches the CNS and eye.** Quantification of infectious virus in the peripheral organs, CNS and eye at 9 dpi monitored by **A)** Plaque assay and **B)** $TCID^{50}$ Assay. Data is presented as PFU or TCID ^{50} /mL. **C)** DENV2 RNA was measured using quantitative real-time PCR specific for DENV2 envelope region. Values are presented as presented as the number of viral RNA copies/µg of RNA. (n= ≥ 4 per organ)

Time course of DENV2 infection in the CNS and eye

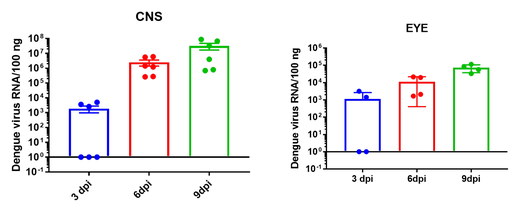
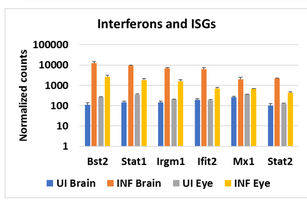
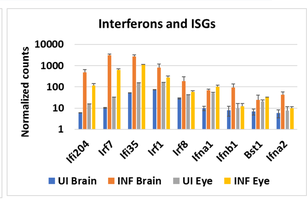
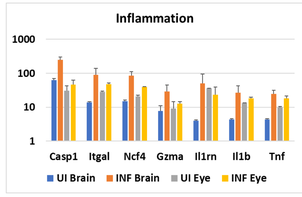


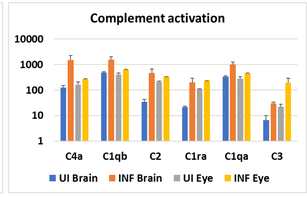
Fig. 3. Time course of DENV2 RNA levels in the CNS and eye. Quantification of DENV2 RNA in the CNS and eye at 3, 6 and 9 dpi was monitored. DENV2 RNA was measured using quantitative real-time PCR specific for DENV2 envelope region. Values are presented as the number of viral RNA copies/100 ng of RNA. n ≥ 4 per time point

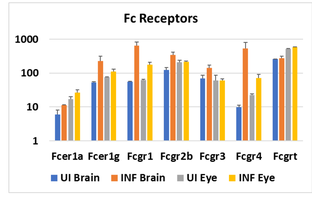
Gene expression profile in CNS and eye of DENV2 infected mice

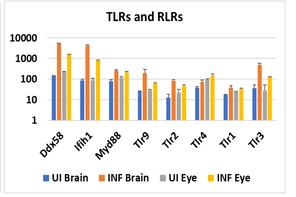


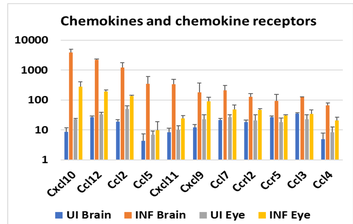


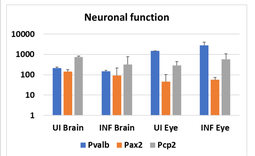


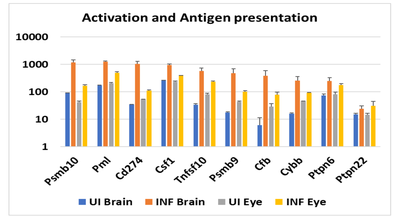












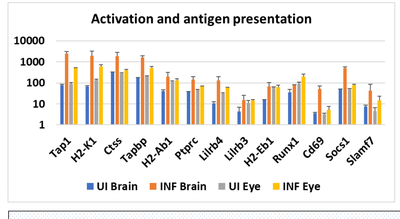
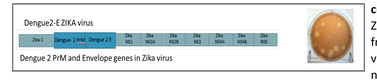


Fig. 4. Gene expression profile in the CNS and eye of DENV2 infected mice. RNA was isolated from the brain and eye of infected mice 6dpi (n ≥ 4 per infected organ and n=2 for uninfected controls) time point using Trizol following manufacturers protocol. NanoString ncounter human immunology panel was used and manufacturers protocol was followed for the study. Data collection was carried out with ncounter digital analyzer to count individual fluorescent barcodes and quantify target RNA molecules present in each sample. Normalized counts of RNA molecules for each gene in the control and infected CNS and eye are presented. The genes analyzed are organized by functional classes. Interferon and ISGs, complement activation, Fc receptors, Inflammation, TLRs and RLRs, Activation and antigen presentation, chemokines and chemokine receptors and Neuronal function.

DENV2 envelope and PrM are sufficient to infect the CNS and eye



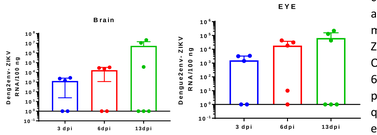


Fig. 5. DENV2 envelope and prm genes are critical for causing disease. A molecular clone Zika virus where the envelope and prm genes from DENV2 and the other genes are from Zika virus (PRVABC59) was constructed. This clone named Deng2env-ZIKV was used to infect c6/36 insect cells, virus was plaque purified and a stock was prepared to infect mice. B6-wt mice infected SC with 2x10^3 PFU of Dengenv-ZIKV at p1 showed virus in the CNS and eye. Quantification of RNA in the CNS and eye at 3, 6 and 13 dpi was monitored (n ≥ 5 per time point). DENV2 RNA was measured using quantitative real-time PCR specific for DENV2 envelope region. Values are presented as the number of viral RNA copies/100 ng of RNA

Conclusion

- ✓ **Infection of immunocompetent neonatal B6 wt mice with DENV2 (Dengue virus serotype 2, New Guinea C strain) results in systemic infection, reaches the CNS and eye and results in neurological symptoms including tremors and hyperactivity and results in death in 50% of infected mice.**
- ✓ **2. DENV2 RNA and infectious virus can be transiently detected in the periphery but persistent in the CNS and eye even after clearance from the periphery.**
- ✓ **3. Gene expression in the CNS and eye of infected mice show inflammation and innate immune response that correlates with the level of infection. Some infected mice that do not show DENV2 RNA at 6 and 9dpi still show signs of inflammation indicating past or ongoing infection.**
- ✓ **4. Infection of neonatal mice with Deng2env-ZIKV show that envelope and prm genes are critical for the tropism exhibited in this mouse model of DENV2 virus disease.**
- ✓ **5. This mouse model will be used to understand Antibody Dependent Enhancement of dengue virus disease.**

FDA Mission Relevance Statement

Dengue virus mouse model that we have developed will help product developers and reviewers to test the efficacy of immunomodulatory and anti-DENV therapeutics and to improve our understanding of the determinants of increased disease severity following a heterologous DENV infection or vaccine.

Thanks

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