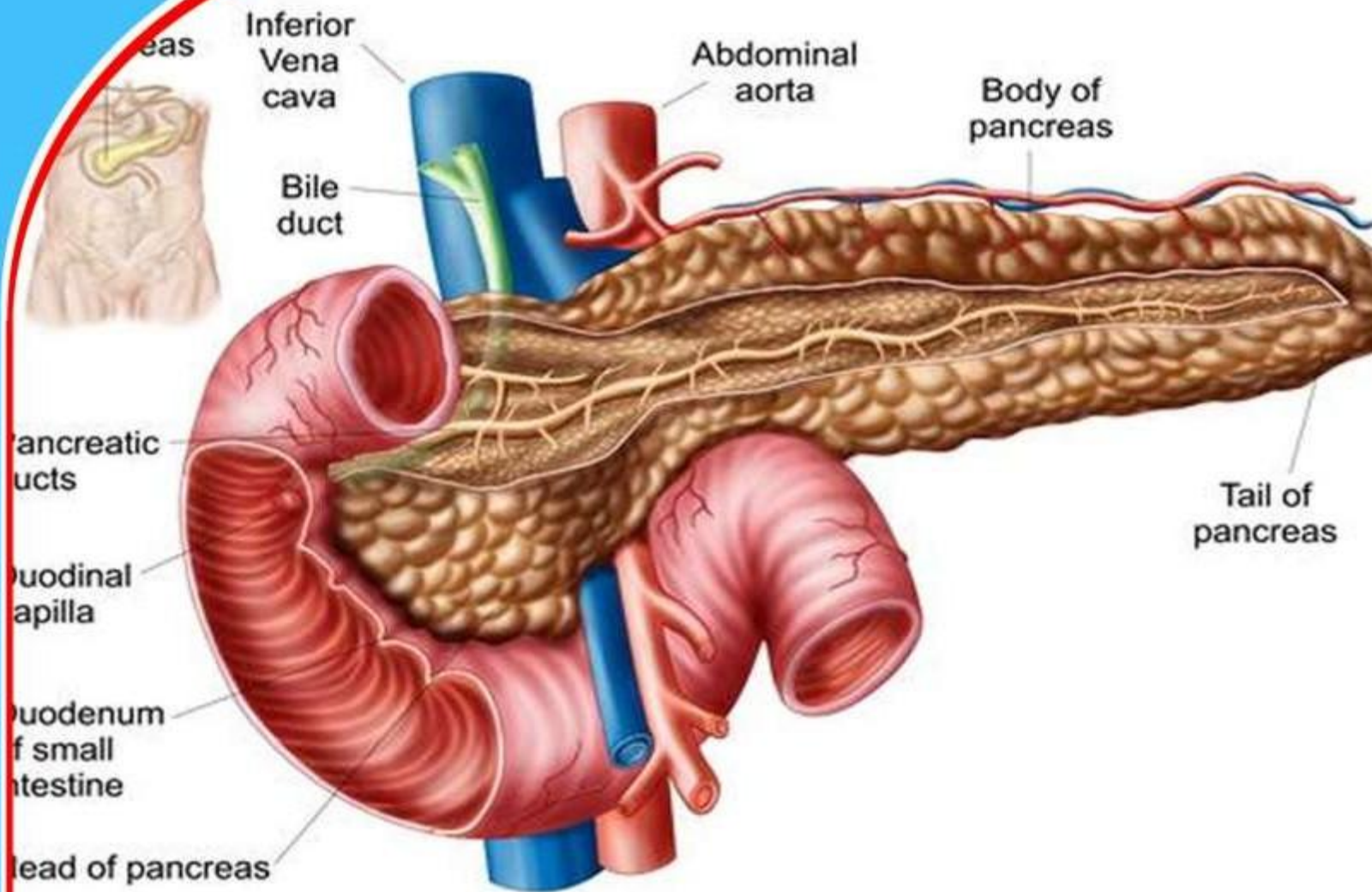


European Journal of Biology (EJB)



Pathogenesis of Dengue virus in Host immune
system and its genomic variation

*Shehreen Sohail, Mukarram Farooq, Fareeha Sohail,
Hamza Rana, Husnain Karim, Tousif Haider, Ahmed
Shakir, Mahrukh Zafar and Samrah Saadat*



Pathogenesis of Dengue virus in Host immune system and its genomic variation

¹Shehreen Sohail, ²Mukarram Farooq, ³Fareeha Sohail, ⁴Hamza Rana, ⁵Husnain Karim,

⁶Tousif Haider, ⁷Ahmed Shakir, ⁸Mahrukh Zafar, ⁹Samrah Saadat

¹University of Central Punjab, MS Microbiology

^{2,4,9}Allama Iqbal Medical College, MBBS

³Government College University, BS Microbiology

^{5,6}University of Central Punjab, MS Microbiology

⁷University of Lahore, MS Forensic

⁸University of Central Punjab, BS Biotechnology

Corresponding Author's Email: shehreen.baig19@gmail.com

Abstract

Dengue viruses are the most prevalent arthropod-borne viral diseases in humans, infecting 50-100 million people each year. Its serotypes are the most common causes of arboviral illness, putting half of the world's population at risk of infection. Because there is no vaccine or antiviral medicines, the only way to manage the disease is to reduce the *Aedes* mosquito vectors. DENV infection can be asymptomatic or cause a self-limiting, acute febrile illness with varying degrees of severity. High fever, headache, stomach discomfort, rash, myalgia, and arthralgia are the typical symptoms of dengue fever (DF). Thrombocytopenia, vascular leakage, and hypotension are symptoms of severe dengue, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Systemic shock characterizes DSS, which can be deadly. Dengue virus infection pathogenesis is linked to a complex interaction between virus, host genes, and host immune response. Major drivers of disease vulnerability include host factors such as antibody-dependent enhancement (ADE), memory cross-reactive T cells, anti-DENV NS1 antibodies, autoimmunity, and genetic variables. The NS1 protein and anti-DENV NS1 antibodies were thought to be involved in the development of severe dengue. The progressive infection may change the cytokine response of cross reactive CD4+ T cells. The need for dengue vaccines that can generate strong protective immunity against all four serotypes is required. To create such vaccines, a thorough understanding of DENV adaptive immunity is required. Structural and functional research have shown that the degree of prM protein cleavage as well as the ensemble of conformational states sampled by virions influence DENV sensitivity to antibody-mediated neutralization, which has crucial implications for vaccine formulation.

Keywords: *Dengue virus, Vector, Host immune system, antibody dependent enchantment, genomic variation.*

Introduction

Dengue fever is a viral disease caused by vector borne dengue viruses (DENVs), which are divided into four serotypes (DENV 1–4) and are members of the Flaviviridae family, genus flavivirus [1]. The World Health Organization (WHO) estimates that around 100 million globally individuals are infected each year, with approximately 500,000 people suffering from dengue hemorrhagic fever (DHF). DHF can progress to dengue shock syndrome (DSS), which has a 12.5 percent fatality rate. Although DENV infections might be asymptomatic, the four DENV serotypes can cause a wide range of illnesses in humans. The illnesses range in severity from nonspecific acute inflammatory sickness to the potentially fatal syndromes DHF/DSS [2]. Classical DF manifests itself with a sudden onset of high fever after an incubation period of 3 to 15 days. Dehydration during the fever phase can induce neurological abnormalities and febrile seizures in young children [3]. Dengue fever is a severe sickness resulting in headache, retro-orbital discomfort, myalgia, arthralgia, petechiae rash, and leucopenia characterizes the syndrome, which is self-limiting. 3-5 days after the beginning of fever, a maculopapular recovery rash emerges [4]. Due to the un survivable symptoms of acute muscle and joint pain lasting 7 days, DF is frequently referred to as "break bone fever." Early signs of DF and DHF are similar, but DHF is linked with hemorrhagic features, including plasma leakage caused by increased vascular permeability, and thrombocytopenia (B100, 000 platelets/mm³). Minor bleeding may occur in moderate infections, which can be severe in people with peptic ulcer disease, and thrombocytopenia is not always limited to severe dengue [5]. Hemoconcentration, ascites, or pleural effusion are symptoms of plasma leakage. The presence of cardiovascular compromise, which occurs when plasma leakage into the interstitial spaces resulting in shock, distinguishes DSS from DHF. A quickly increasing hematocrit, severe stomach pain, prolonged vomiting, and constricted or nonexistent blood pressure are also common clinical warning symptoms for DSS [6].

Dengue fever has been reported to be prevalent in over 100 countries like Pakistan, India, Indonesia, Nepal, Maldives with about 96 million affected persons exhibiting different degrees of severity [7,8]. Dengue fever is a major cause of morbidity and economic burden in many parts of the world, particularly Southeast Asia and the Indian subcontinent [9]. Dengue fever is a mosquito-borne Flavivirus illness spread by *Aedes aegypti*, *Aedes albopictus*, and other *Aedes* species [7,10]. Dengue virus has four antigenically different serotypes, which are DENV-1, DENV-2, DENV-3, and DENV-4 [11]. In October 2013, a new serotype (DENV-5) was discovered in Malaysia's Sarawak state utilizing isolation and genetic sequence analysis [12]. Dengue virus infection takes 4–7 days to develop. Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are among the diseases that can cause symptoms ranging from asymptomatic infection to moderate burning sickness [13]. Coagulation problems, plasma leakage, and enhanced vascular fragility are also present. Fluid loss caused by enhanced capillary permeability results in hypovolemic shock and multi-organ failure. Dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) were the three classifications used by the WHO until 2008 [15]. The current World Health Organization 2009 case categorization system divides symptomatic cases into three categories: dengue without warning symptoms, dengue with danger signs, and dengue hemorrhagic fever [16, 17].

DENV maturation process

An enveloped virus with a positive-sense single-stranded RNA genome that encodes a single polyprotein that is co- and post-translationally processed by viral and different structural proteins capsid (C) protein, host proteases into three precursor membrane (prM) or membrane (M) protein, and envelope (E) protein and seven non-structural proteins termed as NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The C protein binds to the viral DNA, producing a nucleocapsid enveloped by a host lipid bilayer into which the prM and E proteins are attached in immature virions along with the M and E proteins are embedded in mature virions as shown in (Figure 1). The mature dengue virion's cryo-electron microscopy (cryo-EM) structures revealed a smooth surface composed of 180 copies of the M and E proteins, which are linked to the underlying lipid bilayer via their transmembrane helices (Figure 1b). The surface proteins and molecules are arranged in a pseudo-icosahedral fashion, showing 60 asymmetric units made up of three pairs of M and E proteins. An asymmetric unit's three unique E proteins reside in separate chemical environments determined by their closeness to the two-, three-, or five-fold corners [18, 19, 20,21]. The monomer of E protein is made up of three structural domains (EDI, EDII, and EDIII), each with two N-linked glycosylation sites (Asn67 and Asn153) [22], and two among these protomers form a head-to-tail homodimer [23, 24, 25]. Three E protein dimers form a bridge by lying parallel to each other, and 30 of these rafts are organized in a distinctive ' pattern. Virus adherence to myeloid lineage cells is considered to occur via EDIII [26, 27] and the Asn67-linked glycan in EDII [28, 29].

Endosome acidification causes E protein dimers to dissolve and rearrange into trimers, exposing the hydrophobic fusion loop (FL) of EDII at their tips [30, 31]. The FL subsequently inserts into the endosomal membrane, causing the viral and endosomal membranes to fuse and the nucleocapsid to be delivered into the cytoplasm [32]. As shown in figure 1a, dengue virion has a rough surface with 60 spikes which is considered immature generated by three prM–E heterodimers in which the precursor (pr) peptide of the prM protein covers the FL found at the distal end of each E protein monomer [33,34]. The low-pH environment of the trans-Golgi network (TGN) during export causes prM–E heterodimers to reorganize into a folded orientation, exposing a cleavage site at the pr-M junction that is recognized by the host protease furin [35,35]. The neutral pH of the extracellular environment causes the pr peptide to dissociate from the viral particle, ending the viral replication maturation process. Infected cells typically secrete a complex combination of totally immature, partially mature, and fully mature virions as a result of inadequate prM protein cleavage. The effectiveness of all this cleavage process appears to vary between cell types, since viral particles produced from primary human cells, such as dendritic cells, have considerably lower amounts of prM protein than those obtained from insect or mammalian cell lines (e.g., Vero cells). Because the presence of prM protein inhibits the structural rearrangement of E proteins necessary for membrane fusion, fully immature virions are intrinsically non-infectious [35,36,37,38]. Partially mature virions, on the other hand, have two distinct areas of immature and mature structure, with the latter lacking prM protein and therefore possibly capable of beginning infection [39].

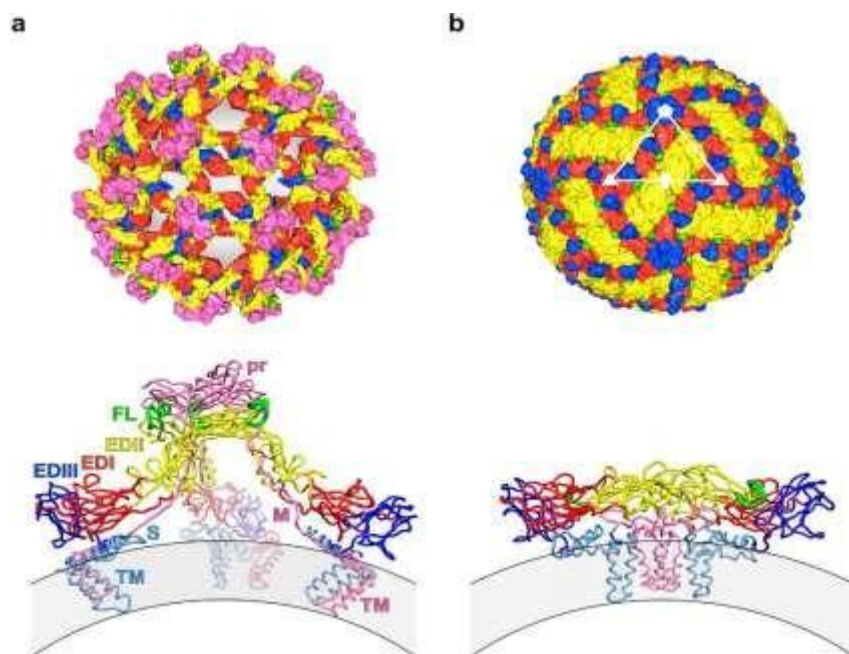


Figure 1: Upper panel: Cryo-electron microscopy structure of the immature dengue virus 1 (DENV1) particle carrying 60 trimeric precursor membrane (prM)–E spikes (PDB 4B03) in surface representation. Lower panel: Side view of a single trimeric prM–E spike in ribbon form. **(b)** Upper panel: Cryo-EM structure of the mature DENV1 particle with 90 E protein dimers (PDB 4CCT) in surface representation.

Source: https://www.researchgate.net/profile/Lucas-Wilken/publication/342170783/figure/fig15/AS:902629068652548@1592214948949/Structural-architecture-of-immature-and-mature-dengue-virions-a-Upper-panel_W640.jpg.

Replication and pathogenesis

DENV is a single-stranded positive-sense RNA virus that is enveloped. The RNA genome is around 10,700 nucleotides long and encodes a 3,411 amino acid long precursor polyprotein with three structural proteins those are capsid [C], precursor membrane [prM], and envelope [E] and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The protein molecules are components of the mature virus particle, whereas the NS proteins are expressed exclusively in the infected cell and are not packed into mature particles in detectable amounts. The structural proteins are not involved in viral genome replication [40,41]. Similar to biological mRNA, the 5'-end has a type I cap, and viral RNA (vRNA) is translated by a cap-dependent initiation scanning the 5'-UTR. The 3'-end lacks a poly(A) tail but terminates in a stem-loop (SL) structure that is preserved. For effective translation and replication, both the 5'- and 3'-UTRs are necessary [42,43]. The UTRs contain unique secondary structures that impart diverse roles and shows better sequence conservation across DENV serotypes. A long stem-loop (SLA) in the 5'-

UTR is thought to function as a promoter for the viral RNA-dependent RNA polymerase NS5 [44]. The 5'- and 3'-UTRs have complementary AUG Regions (UAR) and cyclization sequences (CS), which hybridize to regulate genomic nucleophilic attack and RNA production [42]. Virions attach to cell-surface attachment molecules and receptors and are absorbed by endocytosis during the various stages of the flavivirus life cycle. Due to the low pH of the endosome, viral glycoproteins mediate the fusing of viral and cellular membranes, allowing the virion to disassemble and release vRNA into the cytoplasm. The viral NS proteins replicate the genomic RNA after vRNA is translated into a polyprotein that is processed by viral and cellular proteases. Virus particles are formed at the endoplasmic reticulum (ER) membrane, where C protein and vRNA are encapsulated by the ER membrane and glycoproteins. Finally, immature virus particles are carried via the secretory route, where furin-mediated cleavage of prM causes viral maturation in the acidic environment of the trans-Golgi network (TGN). The mature virus is transmitted from the cell. Before entering the cell by clathrin-mediated endocytosis, DENV viruses attach to host cell factors, its envelopes protein-mediated fusing of viral and cellular membranes occurs in response to complexation, following transportation to endosomal regions, permitting virus particle disintegration and release of single-stranded viral RNA into the cytoplasm, proceeding translation. a polyprotein is a region where viral RNA begins translation, which is further digested by cellular and viral proteases. The viral RNA is subsequently replicated by nonstructural (NS) proteins. The construction of viral particles takes place on the membrane of the endoplasmic reticulum (ER), and the particles then bud into the ER as juvenile virus particles. Pre-membrane (prM) protein is broken by the cellular serine protease furin during the egress of the progeny virus particle through the secretory route. Virus particles that have matured are discharged into the extracellular space. The red inset depicts the viral proteins' probable membrane structure. TGN stands for trans-Golgi network.

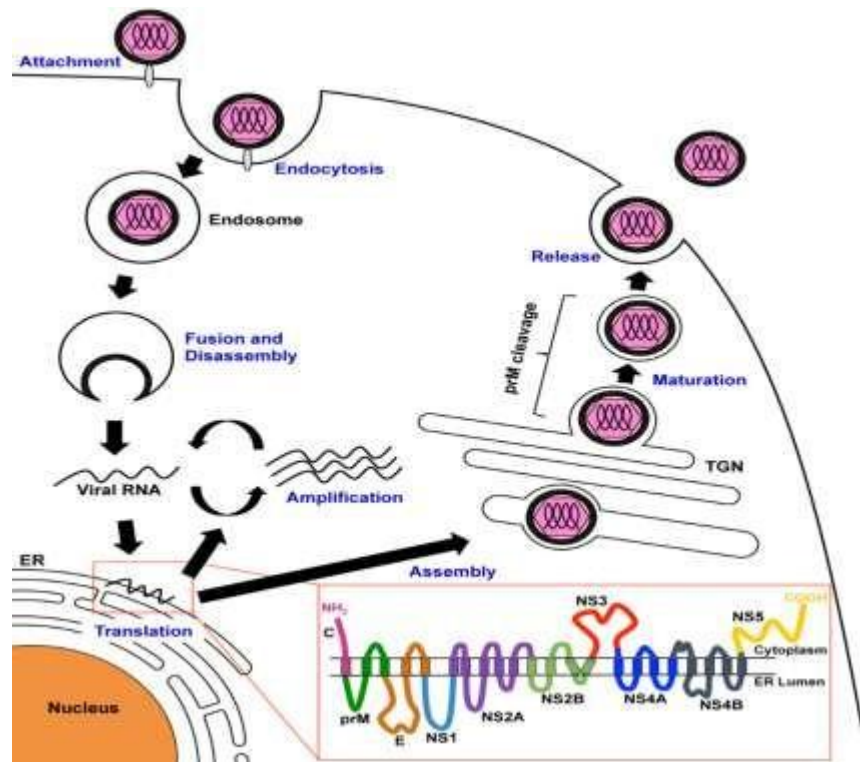


Figure 2: The dengue virus (DENV) life cycle.

Source: https://www.mdpi.com/viruses/viruses-08-00122/article_deploy/html/images/viruses-08-00122-g001-1024.png.

Laboratory diagnosis

The diagnosis which laboratory-based is frequently unavailable at the point of treatment, the initial diagnosis is based on a combination of travel history and clinical symptoms. Because DENV incubation time is shorter than 2 weeks, travel history gives critical information that can rule out other potentially life-threatening illnesses [45]. Culture of the virus, polymerase-chain reaction (PCR), or serologic tests are used to confirm a DENV infection. However, each test has limits, and detection is dependent on various NS1 protein is detectable in serum by enzyme-linked immunosorbent assay (ELISA) from the first day of fever up to 9 days post-infection [46]. NS1-based ELISAs have emerged as a valuable diagnostic tool for acute samples in which IgM is not detected and PCR is not accessible. There are several commercial NS1 antigen kits available, and they are frequently used in both endemic and non-endemic countries.

DENV Genome Variation

Sub genomic and genomic RNA All four DENV serotypes have been linked to severe dengue illness. However, because of genetic variations across DENV genotypes, each genotype has a different virulence and pandemic potential. Few genotypes and strains lead to the development of

severe clinical illness [47,48]. The dendritic cells seen in the epidermis which are initially immature. This gives it a selection advantage over viruses with lesser pathogenic potential. The production of negative-strand RNA during early infection may be responsible for increased viral output. This increases the amount of genomic RNA and the quantity of infectious viral particles released. As a result, Southeast Asian viruses are better suited for viral offspring generation, regardless of the number of cells infected. The Phylogenetic research on several DENV-2 genotypes revealed that the native American genotype was linked with moderate illness, but the introduction of the Southeast Asian genotype in four separate nations corresponded with the emergence of DHF [49,50]. The variation in the DENV genome is not the contributing to the pathophysiology of severe dengue; sub-genomic flavivirus RNA (sfRNA) also plays a vital role in DENV replication in host immune system cells. sfRNA is produced during replication when the DENV RNA genome (11 kb) is incompletely broken down into short RNA (0.3–0.5 kb) by host exoribonuclease [51,52,53,54]. This sfRNA accumulates and inhibits host antiviral immune responses, namely type-1 IFN signalling [55]. RNA interference (RNAi) is a key host defensive mechanism against viral infections. NS4B's involvement in interfering with RNAi pathways, sfRNA plays a role. It binds to the Dicer protein and prevents dsRNA cleavage to small interfering RNA (siRNA). DENV sfRNA binds to the Tripartite motif-containing protein 25 (TRIM25) and prevents ubiquitination-mediated RIG-I activation, suppressing IFN production [56,57]. Secondary structure of the DENV 3'UTR revealing the position and size of sfRNA discovered by sequencing research. Plots depicting the relative quantities of sfRNA species generated by human or mosquito infected cells are shown below in figure 3. Parts B and C show Northern blot hybridization with particular probes complementary to the viral 3'UTR using RNA collected at 30 and 50 hpi from C6/36 or A549 cells infected with DENV-M or DENV-H stocks, respectively. DENV sfRNA were found in infected *Aedes albopictus* and *Aedes aegypti* mosquitos. Northern blot hybridization using RNA isolated from infected mosquitos with DENV-M or DENV-H. For detection, a probe complementary to the viral 3'SL was employed.

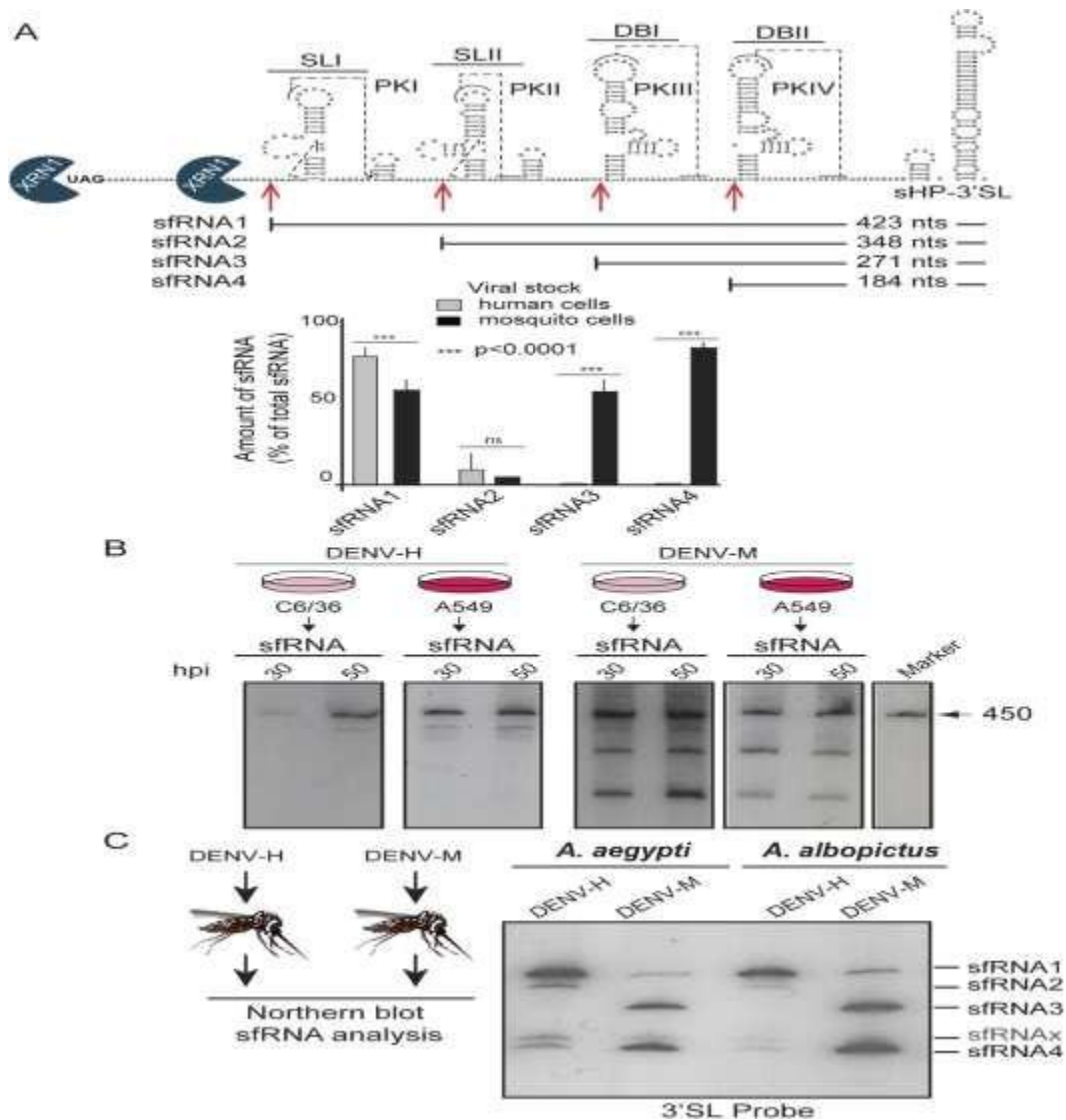


Figure 3: Dengue virus genomic variation associated with mosquito adaptation defines the pattern of viral non-coding RNAs and fitness in human cells

Source:

<https://journals.plos.org/plospathogens/article/file?id=10.1371/journal.ppat.1006265.g002&type=large>.

Conclusion

DENV is a Flavivirus spread by mosquitos that is endemic in many tropical and subtropical regions. The NS proteins are in charge of viral replication as well as host innate immune evasion. The innate immunological response to DENV is poorly understood, as are the precise functions of the NS proteins in immune evasion. Viral replication and host innate immune evasion is processed by NS proteins. The innate immunological response to DENV is poorly understood, as are the functions of the NS proteins in immune evasion. The major innate immune response is type I IFN, and the virus's principal evasion method is to target the type I IFN response. Complement activation, apoptosis, autophagy, and RNAi are examples of innate immune responses. In order to create effective antivirals, it is critical to understand the host's innate immune response to infection and how the virus avoids or exploits it. There is apparently just one approved vaccine, Sanofi-Dengvaxia, Pasteur's which employs the prM-E dengue sequence in a yellow fever viral backbone. The vaccine's delivery has been highly criticized since it has only approximately 60% effectiveness [58]. There are no authorized DENV antivirals. To develop an effective vaccine or antiviral against DENV, it is important to first understand the function of the innate immune response to DENV. Paracetamol for high fever and oral or intravenous fluid intake are treatments for clinical symptoms of acute febrile illness. Recent DENV antiviral research has concentrated on finding new drugs that target the DENV proteins involved in replication and innate immune evasion. NITD-618, a chemical targeting NS4b, suppresses RNA synthesis in vitro in all four serotypes of DENV91 that has neutralizing effect against DENV, targeting the viral envelope lipid bilayer [59].

Recommendations

There is currently an urgent need for dengue vaccines that can generate strong protective immunity against all four serotypes, regardless of DENV serostatus at the time of immunization. To create such vaccines, a thorough understanding of DENV adaptive immunity is required. Structural and functional research have shown that the degree of prM protein cleavage as well as the ensemble of conformational states sampled by virions influence DENV sensitivity to antibody-mediated neutralization, which has crucial implications for vaccine formulation. Antibodies that recognize epitopes on virions that are seldom exposed, such as the FLE, are more likely to increase rather than neutralize infection, and so their annotation by vaccines should be avoided. Vaccine-induced antibody responses should be directed to antigenic regions available in all known DENV configurations, namely EDIII and the EDE, allowing efficient neutralization at various phases of infection. More research is being done to develop strategies for selectively inducing neutralizing antibody responses to these epitopes. Furthermore, new appropriate tests for evaluating vaccination performance must be developed. The CYD-TDV studies revealed that vaccine-induced antibodies' capacity to neutralize cell culture-derived virions was very weakly linked to their in vivo effectiveness [60]. Data from immunological examinations of DENV-infected patients supplement the findings of mice investigations. A recent report of recurrent DENV3 infection in a lymphogenic kidney transplant patient revealed that, despite regularly detectable levels of neutralizing antibodies, infection clearance occurred only after CD8⁺ T-cell counts climbed to the lower normal range [61]. Both activation of canonical signalling pathways and expression of surface markers associated with an activated T-cell phenotype were enhanced in

asymptomatic viremic people compared to clinical dengue patients in a pediatric cohort [62]. Another study found that the increase of CD8⁺ effector memory T-cell subsets was associated with lower virus loads in acute dengue patients [63]. Furthermore, the presence of IFN-producing DENV-specific T cells in dengue patients was found to be related with milder clinical illness and viraemia resolution [64]. New discoveries on the role of NS1 [65] in dengue pathogenesis and its capacity to produce protective immune responses have rekindled interest in employing this protein as a vaccine candidate, in part because it is not included in the already approved dengue vaccine. The presence of various conserved epitopes on NS1 and the cross-inhibitory effects of NS1 antisera found in animal models [66, 67] imply that a single immunogen may provide protection against different serotypes. The fundamental challenge in creating NS1-based vaccines is the danger of inducing vascular disease by NS1 treatment alone or the subsequent generation of innate immune antibodies.

References

1. Westaway EG, Brinton MA, Gaidamovich S, Horzinek MC, Igarashi A, Kaariainen L, et al. Flaviviridae. Intervirology 1985; 24: 18392
2. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol 2002; 10: 1003.
3. WHO Dengue: guidelines for diagnosis, treatment, prevention and control new ed. Geneva: World Health Organization; 2009.
4. Henchal EA, Putnak JR. The dengue viruses. Clin Microbiol Rev 1990; 3: 37696
5. Tsai CJ, Kuo CH, Chen PC, Changcheng CS. Upper gastro-intestinal bleeding in dengue fever. Am J Gastroenterol 1991; 86: 33-5.
6. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol 2002; 10: 100-3.
7. Mutheneni SR, Morse AP, Caminade C, Upadhyayula SM (2017) Dengue burden in India: recent trends and importance of climatic parameters. Emerg Microbes Infect 6(8): e70
8. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL et al (2013) The global distribution and burden of dengue. Nature. 496(7446):504–507.
9. Hussain T, Jamal M, Rehman T, Andleeb S (2015) Dengue: pathogenesis, prevention and treatment – a mini review. Adv Life Sci 2(3):110–114.
10. Hermann LL, Gupta SB, Manof SB, Kalayanarooj S, Gibbons RV, Collier B-AG (2015) Advances in the understanding, management, and prevention of dengue. J Clin Virol 64:153–159.
11. Holmes EC (1998) Molecular epidemiology and evolution of emerging infectious diseases. Br Med Bull 54(3):533–543.
12. Mustafa MS, Rasotgi V, Jain S, Gupta V (2015) Discovery of fifth serotype of dengue virus (DENV-5): a new public health dilemma in dengue control. Med J Armed Forces India 71(1):67–70.
13. Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustafa AS (2000) Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. FEMS Immunol Med Microbiol 28(3):183–188.
14. Pang X, Zhang R, Cheng G (2017) Progress towards understanding the pathogenesis of dengue hemorrhagic fever. Virol Sin 32(1):16–22.
15. World Health Organization (1997) Dengue hemorrhagic fever diagnosis, treatment, p.pdf [Internet]. [cited 2018 July 3].
16. Mathew A, Rothman AL (2008) Understanding the contribution of cellular immunity to dengue disease pathogenesis. Immunol Rev 225:300–313
17. Guzman MG, Alvarez M, Halstead SB (2013) Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. Arch Virol 158(7):1445–1459.
18. Kuhn, R.J.; Zhang, W.; Rossmann, M.G.; Pletnev, S.V.; Corver, J.; Lenches, E.; Jones, C.T.; Mukhopadhyay, S.; Chipman, P.R.; Strauss, E.G.; et al. Structure of dengue virus: Implications for flavivirus organization, maturation, and fusion. Cell 2002, 108, 717–725. [CrossRef]

19. Zhang, X.; Ge, P.; Yu, X.; Brannan, J.M.; Bi, G.; Zhang, Q.; Schein, S.; Zhou, Z.H. Cryo-EM structure of the mature dengue virus at 3.5-Å resolution. *Nat. Struct. Mol. Biol.* 2013, 20, 105–110. [CrossRef]
20. Kostyuchenko, V.A.; Zhang, Q.; Tan, J.L.; Ng, T.-S.; Lok, S.-M. Immature and mature dengue serotype 1 virus structures provide insight into the maturation process. *J. Virol.* 2013, 87, 7700–7707. [CrossRef]
21. Kostyuchenko, V.A.; Chew, P.L.; Ng, T.-S.; Lok, S.-M. Near-atomic resolution cryo-electron microscopic structure of dengue serotype 4 virus. *J. Virol.* 2014, 88, 477–482. [CrossRef]
22. Zhang, W.; Chipman, P.R.; Corver, J.; Johnson, P.R.; Zhang, Y.; Mukhopadhyay, S.; Baker, T.S.; Strauss, J.H.; Rossmann, M.G.; Kuhn, R.J.; et al. Visualization of membrane protein domains by cryo-electron microscopy of dengue virus. *Nat. Struct. Biol.* 2003, 10, 907–912.
23. Modis, Y.; Ogata, S.; Clements, D.; Harrison, S.C. A ligand-binding pocket in the dengue virus envelope glycoprotein. *Proc. Natl. Acad. Sci. USA.* 2003, 100, 6986–6991. [CrossRef] [PubMed]
24. Modis, Y.; Ogata, S.; Clements, D.; Harrison, S.C. Variable surface epitopes in the crystal structure of dengue virus type 3 envelope glycoprotein. *J. Virol.* 2005, 79, 1223–1231. [CrossRef] [PubMed]
25. Zhang, Y.; Zhang, W.; Ogata, S.; Clements, D.; Strauss, J.H.; Baker, T.S.; Kuhn, R.J.; Rossmann, M.G. Conformational changes of the flavivirus E glycoprotein. *Structure* 2004, 12, 1607–1618.
26. Chen, Y.; Maguire, T.; Hileman, R.E.; Fromm, J.R.; Esko, J.D.; Linhardt, R.J.; Marks, R.M. Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nat. Med.* 1997, 3, 866–871.
27. Watterson, D.; Kobe, B.; Young, P.R. Residues in domain III of the dengue virus envelope glycoprotein involved in cell-surface glycosaminoglycan binding. *J. Gen. Virol.* 2012, 93, 72–82. [CrossRef] 49. Pokidysheva, E.; Zhang, Y.; Battisti, A.J.; Bator-Kelly, C.M.; Chipman, P.R.; Xiao, C.; Gregorio, G.G.; Hendrickson, W.A.; Kuhn, R.J.; Rossmann, M.G. Cryo-EM reconstruction of dengue virus in complex with the carbohydrate recognition domain of DC-SIGN. *Cell* 2006, 124, 485–493.
28. Pokidysheva, E.; Zhang, Y.; Battisti, A.J.; Bator-Kelly, C.M.; Chipman, P.R.; Xiao, C.; Gregorio, G.G.; Hendrickson, W.A.; Kuhn, R.J.; Rossmann, M.G. Cryo-EM reconstruction of dengue virus in complex with the carbohydrate recognition domain of DC-SIGN. *Cell* 2006, 124, 485–493. [CrossRef] [PubMed]
29. Miller, J.L.; de Wet, B.J.M.; deWet, B.J.M.; Martinez-Pomares, L.; Radcliffe, C.M.; Dwek, R.A.; Rudd, P.M.; Gordon, S. The mannose receptor mediates dengue virus infection of macrophages. *PLoS Pathog.* 2008, 4, e17.
30. Bressanelli, S.; Stiasny, K.; Allison, S.L.; Stura, E.A.; Duquerroy, S.; Lescar, J.; Heinz, F.X.; Rey, F.A. Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. *EMBO J.* 2004, 23, 728–738. [CrossRef] [PubMed]

31. Modis, Y.; Ogata, S.; Clements, D.; Harrison, S.C. Structure of the dengue virus envelope protein after membrane fusion. *Nature* 2004, 427, 313–319.
32. Nour, A.M.; Li, Y.; Wolenski, J.; Modis, Y. Viral membrane fusion and nucleocapsid delivery into the cytoplasm are distinct events in some flaviviruses. *PLoS Pathog.* 2013, 9, e1003585. mature dengue virus at 3.5-Å resolution. *Nat. Struct. Mol. Biol.* 2013, 20, 105–110.
33. Kostyuchenko, V.A.; Zhang, Q.; Tan, J.L.; Ng, T.-S.; Lok, S.-M. Immature and mature dengue serotype 1 virus structures provide insight into the maturation process. *J. Virol.* 2013, 87, 7700–7707.
34. Zhang, Y.; Corver, J.; Chipman, P.R.; Zhang, W.; Pletnev, S.V.; Sedlak, D.; Baker, T.S.; Strauss, J.H.; Kuhn, R.J.; Rossmann, M.G. Structures of immature flavivirus particles. *EMBO J.* 2003, 22, 2604–2613.
35. Stadler, K.; Allison, S.L.; Schalich, J.; Heinz, F.X. Proteolytic activation of tick-borne encephalitis virus by furin. *J. Virol.* 1997, 71, 8475–8481. [CrossRef] [PubMed]
36. Yu, I.-M.; Zhang, W.; Holdaway, H.A.; Li, L.; Kostyuchenko, V.A.; Chipman, P.R.; Kuhn, R.J.; Rossmann, M.G.; Chen, J. Structure of the immature dengue virus at low pH primes proteolytic maturation. *Science* 2008, 319, 1834–1837.
37. Zybert, I.A.; van der Ende-Metselaar, H.; Wilschut, J.; Smit, J.M. Functional importance of dengue virus maturation: Infectious properties of immature virions. *J. Gen. Virol.* 2008, 89, 3047–3051.
38. Randolph, V.B.; Winkler, G.; Stollar, V. Acidotropic amines inhibit proteolytic processing of flavivirus prM protein. *Virology* 1990, 174, 450–458.
39. Plevka, P.; Battisti, A.J.; Junjhon, J.; Winkler, D.C.; Holdaway, H.A.; Keelapang, P.; Sittisombut, N.; Kuhn, R.J.; Steven, A.C.; Rossmann, M.G. Maturation of flaviviruses starts from one or more icosahedrally independent nucleation centres. *EMBO Rep.* 2011, 12, 602–606.
40. Pang X, Zhang M, Dayton AI. Development of dengue virus replicons expressing HIV-1 gp120 and other heterologous genes: a potential future tool for dual vaccination against dengue virus and HIV. *BMC Microbiol* 2001; 1: 28.
41. Alvarez DE, Lodeiro MF, Filomatori CV, Fucito S, Mondotte JA, Gamarnik AV, et al. Structural and functional analysis of dengue virus RNA. *Novartis Found Symp* 2006; 277: 120-32; discussion 132-5, 251-3.
42. Alvarez DE, Lodeiro MF, Filomatori CV, Fucito S, Mondotte JA, Gamarnik AV, et al. Structural and functional analysis of dengue virus RNA. *Novartis Found Symp* 2006; 277: 120-32; discussion 132-5, 251-3.
43. Gamarnik A. Role of the dengue virus 5' and 3' untranslated regions in viral replication. In: Hanley KA, Weaver SC, eds. *Frontiers in dengue virus research*. Norfolk, UK: Caister Academic Press; 2010, pp. 55-78.
44. Yu L, Nomaguchi M, Padmanabhan R, Markoff L. Specific requirements for elements of the 5' and 3' terminal regions in flavivirus RNA synthesis and viral replication. *Virology*

- 2008; 374: 170-85.
45. Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV, et al. Dengue and dengue haemorrhagic fever. *Lancet* 1998; 352: 971-7.
 46. Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *J Infect Dis* 2002; 186: 1165-8.
 47. Ubol S, Halstead SB (2010) How innate immune mechanisms contribute to antibody-enhanced viral infections. *Clin Vaccine Immunol* 17(12):1829–1835
 48. Mammen MP, Lyons A, Innis BL, Sun W, McKinney D, Chung RCY et al (2014) Evaluation of dengue virus strains for human challenge studies. *Vaccine*. 32(13):1488–1494.
 49. Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, de Chacon et al (1999) Dengue virus structural differences that correlate with pathogenesis. *J Virol* 73(6):4738–4747
 50. Rico-Hesse R, Harrison LM, Salas RA, Tovar D, Nisalak A, Ramos C et al (1997) Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology*. 230(2):244–251.
 51. Pang X, Zhang R, Cheng G (2017) Progress towards understanding the pathogenesis of dengue hemorrhagic fever. *Virol Sin* 32(1):16–22.
 52. Funk A, Truong K, Nagasaki T, Torres S, Floden N, Balmori Melian E et al (2010) RNA structures required for production of subgenomic flavivirus RNA. *J Virol* 84(21):11407–11417
 53. Chapman EG, Costantino DA, Rabe JL, Moon SL, Wilusz J, Nix JC et al (2014) The structural basis of pathogenic subgenomic flavivirus RNA (sfRNA) production. *Science*. 344(6181):307–310.
 54. Roby JA, Pijlman GP, Wilusz J, Khromykh AA (2014) Noncoding subgenomic flavivirus RNA: multiple functions in West Nile virus pathogenesis and modulation of host responses. *Viruses*. 6(2):404–427.
 55. Manokaran G, Finol E, Wang C, Gunaratne J, Bahl J, Ong EZ et al (2015) Dengue subgenomic RNA binds TRIM25 to inhibit interferon expression for epidemiological fitness. *Science*. 350(6257):217–221.
 56. Green AM, Beatty PR, Hadjilaou A, Harris E (2014) Innate immunity to dengue virus infection and subversion of antiviral responses. *J Mol Biol* 426(6):1148–1160
 57. Kao Y-T, Lai MMC, Yu C-Y (2018) How dengue virus circumvents innate immunity. *Front Immunol* [Internet]. [cited 2020 Sep 2];9. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6288372/>.
 58. Aguiar, M., Stollenwerk, N. & Halstead, S. B. The risks behind Dengvaxia recommendation. *Lancet Infect. Dis*. 16, 882 (2016).

59. Aoki-Utsubo, C. et al. Broad-spectrum antiviral agents: secreted phospholipase A 2 targets viral envelope lipid bilayers derived from the endoplasmic reticulum membrane. *Sci. Rep.* 7, 1 (2017).
60. Hadinegoro, S.R.; Arredondo-García, J.L.; Capeding, M.R.; Deseda, C.; Chotpitayasunondh, T.; Dietze, R.; Ismail, H.I.H.M.; Reynales, H.; Limkittikul, K.; Rivera-Medina, D.M.; et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N. Engl. J. Med.* 2015, 373, 1195–1206.
61. Ng, K.-H.; Zhang, S.L.; Tan, H.C.; Kwek, S.S.; Sessions, O.M.; Chan, C.-Y.; Liu, I.D.; Lee, C.K.; Tambyah, P.A.; Ooi, E.E.; et al. Persistent dengue infection in an immunosuppressed patient reveals the roles of humoral and cellular immune responses in virus clearance. *Cell Host Microbe* 2019, 26, 601–605.e3. [CrossRef]
62. Bashyam, H.S.; Green, S.; Rothman, A.L. Dengue virus-reactive CD8⁺ T cells display quantitative and qualitative differences in their response to variant epitopes of heterologous viral serotypes. *J. Immunol.* 2006, 176, 2817–2824. [CrossRef]
63. Imrie, A.; Meeks, J.; Gurary, A.; Sukhbataar, M.; Kitsutani, P.; Effler, P.; Zhao, Z. Differential functional avidity of dengue virus-specific T-cell clones for variant peptides representing heterologous and previously encountered serotypes. *J. Virol.* 2007, 81, 10081–10091. [CrossRef]
64. Simmons, C.P.; Dong, T.; Chau, N.V.; Thi, N.; Dung, P.; Nguyen, T.; Chau, B.; Thi, L.; Thao, T.; Dung, N.T.; et al. Early T-cell responses to dengue virus epitopes in Vietnamese adults with secondary dengue virus infections. *J. Virol.* 2005, 79, 5665–5675. [CrossRef]
65. Hertz, T.; Beatty, P.R.; MacMillen, Z.; Killingbeck, S.S.; Wang, C.; Harris, E. Antibody epitopes identified in critical regions of dengue virus nonstructural 1 protein in mouse vaccination and natural human infections. *J. Immunol.* 2017, 198, 4025–4035. [CrossRef] [PubMed].
66. Beatty, P.R.; Puerta-Guardo, H.; Killingbeck, S.S.; Glasner, D.R.; Hopkins, K.; Harris, E. Dengue virus NS1 triggers endothelial permeability and vascular leak that is prevented by NS1 vaccination. *Sci. Transl. Med.* 2015, 7, 304ra141. [CrossRef]
67. Espinosa, D.A.; Beatty, P.R.; Reiner, G.L.; Sivick, K.E.; Glickman, L.H.; Dubensky, T.W.; Harris, E. Cyclic dinucleotide-adjuvanted dengue virus nonstructural protein 1 induces protective antibody and T cell responses. *J. Immunol.* 2019, 202, 1153–1162. [CrossRef]