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Understanding Dengue Proteins: Key Players in Pathogenesis and Treatment Strategies

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ABSTRACT

The Dengue virus, part of the Flaviviridae family of arboviruses, can lead to a range of clinical outcomes, from no symptoms to severe conditions like dengue hemorrhagic fever and dengue shock syndrome. Despite extensive investigation, finding an effective solution for the disease remains challenging. The virus has a single-stranded RNA genome encoding a polyprotein chain, which is cleaved by viral and cellular proteases to produce 10 distinct viral proteins. Understanding these proteins is crucial for managing dengue. They fall into two categories: structural proteins (C-protein, E-protein, and prM), involved in virus entry, assembly, and secretion within host cells, and non-structural proteins (NS1–5), essential for viral assembly, replication, and immune evasion, influencing the development and severity of dengue fever. Furthermore, this review examines the controversy surrounding Dengvaxia, the only commercially available dengue vaccine, and associated risks, while also exploring various approaches for developing a potent antidote for dengue.

Keywords: *Dengue Virus, Viral Proteins, Anti-Dengue Treatment, Structural and Non-Structural Protein, Dengvaxia, Protein Structure*

INTRODUCTION

The dengue virus is spherical, measuring about 50 nanometers in diameter. Its core architecture comprises viral DNA and C proteins, forming the nucleocapsid, which is shielded by a viral envelope made from host-derived lipid bilayers. Within this envelope, the E and prM proteins are embedded, with 180 copies of each protein spanning the lipid bilayer.These proteins work together to form a protective barrier around the virus, impeding its ability to infiltrate human cells.

Dengue viruses possess a single-stranded RNA with a positive sense as their genetic material. Positive-sense RNA is called this because it can be directly translated into proteins without delay. This virus has a genome that comprises ten genes. The genome is transcribed into a solitary elongated polypeptide and cleaved into 10 discrete proteins. (Figure 1) The ten proteins include the capsid (C), membrane (prM), and envelope (E) and seven non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. (Figure 2)

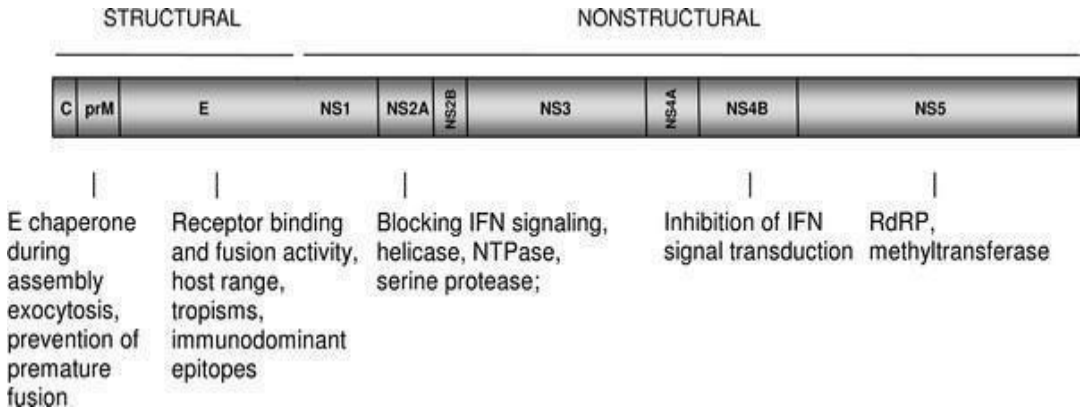

Figure 1: Dengue virus genome map (Adapted and revised from Zybert, 2011).

Below, we will explore the detailed structure of the dengue virus's structural and nonstructural proteins.

STRUCTURAL PROTEINS

CAPSID

The Dengue Virus (DENV) Capsid (C) protein is a crucial structural protein in packaging the viral genome. It interacts with both RNA and lipids. The protein is a fundamental molecule with a molecular weight of 12 kilodaltons9 (kDa) and is present in solution as a dimer. The dimer is made stable by hydrophobic interactions between helices 2 (2-2') and 4 (4-4') that are oriented in opposite directions. Conserved residues, such as tryptophan 69, are crucial in stabilisation (Ma et al., 2004; Byk & Gamarnik, 2016). The N2b-NS3 protease cleaves the C protein, explicitly removing the hydrophobic signal region at the C-terminal before viral assembly (Yamshchikov & Compans, 1995).

The C protein not only plays a structural role but also triggers antiviral responses in CD4+ T-cells and increases interferon production in CD8+ T-cells (Weiskopf et al., 2016). When employed as a vaccine, the C protein elicited antibodies that exhibited antiviral properties, albeit without the ability to produce neutralising antibodies. This led to a survival rate of 44% in mice, as reported by Lazo et al. in 2019. Studies have demonstrated that tetravalent formulations of recombinant C proteins from all serotypes can improve cell-mediated immunity against dengue (Gil et al., 2017). These findings indicate that the C protein shows promise as an antigen in developing a dengue vaccine.

MEMBRANE

A small Dengue virus protein is prM glycoprotein. It weighs 9 kDa and has 75 amino acids (Murray et al., 1993). The mature virus possesses the prM protein on its surface, whereas the immature virus has its precursor (Kuhn et al., 2002). The viral membrane's outer layer contains prM's N-terminus (Zhang et al., 2003). The stem region of prM (amino acids 111–131) interacts with viral membrane E proteins to reorganise surface proteins during virus maturation in low pH exosomes (Zhang et al., 2012). Furin cleaves prM in the last maturation phase (Junjhon et al., 2008).

Human V-ATPase helps Dengue virus enter and exit cells by interacting with prM amino acids 76–80 (Duan et al., 2008). New prM partner claudin-1 is also found. Dengue virus modulates claudin-1 expression to increase viral entry and avoid superinfection (Gao et al., 2010).

ENVELOPE

One of the most essential components of the Dengue Virus is the envelope glycoprotein, (E.) Along with the proteins in the capsid and the membrane, it helps the virus enter host cells by creating a protective coating over the RNA inside. There are 180 E proteins on the surface of a mature DENV particle, and they are arranged in pairs. The binding of the virus to cell surface receptors and the regulation of its antigenicity are both influenced by these

proteins (Mondotte et al., 2007). The E protein has three domains and is around "53 kDa" in size. The nine-stranded beta-barrel structure of Domain I extends across residues 1-52, 132-192, and 281-295. The hydrophobic peptide found in Domain II, which spans residues 53-131 and 193-280, is essential for viral fusion with host cell membrane (Austin et al., 2012). The principal binding and antigenic domain, Domain III, which extends from residues 296-395. (Pierson and Diamond ,2012). Researchers often use the E protein in vaccination research. One example is DNA vaccines that use a plasmid vector (pVR1012) containing prM and E proteins to trigger the formation of antibodies that neutralise DENV (Porter et al., 2012). The DIII domain has a crucial function in initiating the immunological response, which results in the generation of antibodies that provide protection and neutralisation (Fahimi et al., 2018). Studies have shown that vaccinations utilising viral vectors that produce a truncated E protein or only the DIII domain can significantly boost immune response in both mice and macaques (White et al., 2013).

NON-STRUCTURAL PROTEIN-1

The NS1 protein of the Dengue Virus is crucial for viral replication and evading the immune system. NS1 is a protein that has undergone glycosylation and has a molecular mass of around 48 kDa. It can interact with lipids found in cell membranes. NS1 is initially produced as a monomer and later undergoes post-translational changes to become dimers and hexamers. It enhances the process of copying the genetic material of a virus by interacting with the NS4A and NS4B proteins (Alayli & Scholle, 2016). NS1 is involved in releasing cytokines and rupturing the integrity of a layer of endothelial cells, resulting in the leakage of blood vessels and the occurrence of hemorrhagic shock. TLR4 activation is linked to this disease, suggesting that NS1 could be a viable target for therapeutic intervention (Modhiran et al., 2017).

DENV replication is energetically demanding, as NS1 interacts with and stimulates glyceraldehyde-3-phosphate dehydrogenase, increasing glycolysis and energy generation during viral replication (Allonso et al., 2015). The use of NS1-based vaccination techniques is now being studied, with researchers investigating the potential of adjuvants to improve the vaccine's effectiveness (Amorim et al., 2012). Furthermore, studies have demonstrated that anti-NS1 antibodies can effectively inhibit viral replication, lower DENV-induced mortality, and alleviate NS1-mediated pathogenesis, indicating their potential as therapeutic agents (Lai et al., 2017).

NON-STRUCTURAL PROTEIN-2A

Non-structural protein-2A (NS2A) is an important part of the Dengue Virus (DENV), although it doesn't contribute to the virus's structure. It weighs 22 kDa and plays key roles in making viral RNA, assembling new viruses, suppressing the immune response, and forming viral membranes. While its exact structure is not known, research indicates that the first 68 amino acids are located in the endoplasmic reticulum (ER) lumen, followed by five transmembrane segments covering residues 69-209 in the DENV2 region, and a C-terminal section (residues 210-218) in the cytoplasm.

The N-terminal region of NS2A is crucial for RNA replication and the virus's harmful effects on cells (Wu et al., 2017). This region is also involved in viral assembly (Leung et al., 2008). NS2A is essential for RNA synthesis, virus assembly, and maturation (Xie et al., 2015). Its transmembrane domains are vital for viral replication, assembly, and secretion (Wu et al., 2015). Besides its roles in replication and assembly, NS2A helps the virus evade the immune system by downregulating “type I interferon" and contributing to virus-induced cell damage (Liu et al., 2006; Chang et al., 1999). It was also discovered that NS2A epitopes can induce cytotoxic T lymphocyte (CTL) responses, offering significant promise for dengue virus (DENV) vaccine development.These CTL responses have the

potential to greatly improve the efficacy of DENV vaccines. (Green et al., 1997)

NONSTRUCTURAL PROTEIN-2B

Non-structural protein-2B (NS2B) is a "14 kDa " protein in the Dengue Virus (DENV). It plays a major role in the cleavage of viral proteins, working together with NS3 (Brinkworth et al., 1999). NS2B is an essential cofactor enabling NS3 to function as a serine protease. The N-terminal region of NS2B (specifically residues 49–66 of DENV1) plays a vital role in stabilising NS3, which is essential for the replication and infectivity of the virus (Yildiz et al., 2013). In addition, NS2B can oligomerise and impact the permeability of the cell membrane by creating pore-like structures, resulting in cell lysis (Noble et al., 2012).

Three anticipated transmembrane helices structurally define NS2B. The hairpin structure, specifically "β-strands 2 and 3", is located in the "catalytic region" of NS3 and interacts with the substrate. NS2B encircles the NS3 protease domain in a belt-like arrangement (Noble et al., 2012). After position 76, NS2B assumes a disordered structure with intermittent electron intensity, suggesting a variable shape at the C-terminal in solution. NS2B plays a crucial role in activating NS3 when they are together. It helps NS3 adhere to the cell membrane and activate its protease function in the presence of membrane lipids (Clum et al., 1997). NS2B has a role in stabilising the secondary structure and active site of NS3, which in turn regulates its activity (Luo et al., 2015). NS2B/NS3 synergistically promote RNA unwinding by interacting with RNA and augmenting ATPase activity (Rajagopal et al., 2010). In addition, the NS3 protease produces the amino-terminal portion of NS2B (Preugschat et al., 1990).

The NS2B/NS3 protease complex harms human cells by cutting the human adaptor protein "STING", which prevents the synthesis of "type 1 interferon". The mouse form of "STING" does not exhibit this function, which limits the dengue virus replication in mice cells (Aguirre et al., 2012).

Several inhibitors targeting the NS2B/NS3 complex have been discovered. Virtual screening techniques identified NS1 inhibitors, which were then synthesised into derivatives that effectively block the activity of NS2B/NS3 protease (Deng et al., 2012). In addition, a genetically engineered peptide (RC-1) was used to prevent the action of NS2B/NS3, ultimately stopping the replication of DENV2 in Vero cells (Rothan et al., 2012).

NON-STRUCTURAL PROTEIN-3

Non-structural protein-3 (NS3) is a vital part of the Dengue Virus (DENV), helping the virus infect cells, replicate its RNA, and create new virus particles (Swarbrick et al., 2017). It weighs about 68 kDa and has different parts. The N-terminal part works as a serine protease, while the C-terminal part contains sections for ATP-dependent RNA helicase and RNA triphosphatase. The protease part is made of β-sheets, while the C-terminal parts have both β-sheets and α-helices. There's a flexible linker connecting these sections, allowing for flexibility in structure. NS3 also has a hydrophobic loop called the "GLFG region", important for interacting with the cell membrane (Noble et al., 2012).

NS3, with the help of host proteases, chops up the DENV polyprotein into separate functional proteins (Fields et al., 2007). Its serine protease function needs it to team up with NS2B. Together, they have specific spots where they cut the polyprotein into pieces (Teo & Wright, 1997). Designing an inhibitor targeting the NS3 protease aims to impede its active site, inhibiting its activity (Leung et al., 2001). Several peptide-based inhibitors of the NS3/NS2B protease, both linear and nonlinear, have been documented.

NON-STRUCTURAL PROTEIN-4A

Non-structural protein-4A (NS4A) is a "16 kDa" protein that is not part of the structural components of the cell. It is hydrophobic in nature and plays a vital role in the infection caused by the Dengue virus. NS4A accomplishes this by crossing the endoplasmic reticulum (ER) membrane, a

cellular organelle involved in protein synthesis and lipid metabolism (Miller et al., 2007). The protein structure is divided into two sections: the N-terminal segment is created when NS3 cleaves the viral polyprotein, and the C-terminal segment is produced by an unidentified host signalase (Cahour et al., 1992). NS4A is directed to the endoplasmic reticulum (ER) lumen by a short signal peptide called 2K, which has a mass of around 2 kDa (Lin et al., 1993).

The N-terminal portion of NS4A, consisting of residues 1–48, comprises an amphipathic helix (AH) that exhibits favourable interactions with lipid membranes. Alterations in this specific area, such as L6E and M10E, decrease AH's attraction to lipid membranes, impeding the replication of viruses and the formation of NS4A oligomers (Li et al., 2018). The oligomerisation of NS4A is crucial for virus replication (Lee et al. in 2015). NS4A also triggers P13-dependent autophagy, which safeguards cells against apoptosis and creates a conducive environment for Dengue virus replication (Mclean et al., 2011).

The N-terminus AH, which binds to the cellular protein vimentin, and removing the C-terminal 2K section, which modifies the membrane, have essential functions in viral replication (Teo & Chu, 2014). In addition, NS4A suppresses the immune system's interferon responses, which helps the virus to cause disease and reproduce (Jones et al., 2005). NS4A plays a significant role in both viral replication and the regulation of the host's immunological responses, making it a very intriguing target for suppression.

NONSTRUCTURAL PROTEIN-4B

Non-structural protein-4B (NS4B) is a "hydrophobic protein" consisting of 248 amino acids and weighing approximately "27 kDa". It is located in the endoplasmic reticulum (ER) and spans nine transmembrane domains (Miller et al., 2006). Glycosylation occurs at specific residues (58 and 62) and is essential for RNA replication. Mutations in these residues disrupt this process (Naik & Wu, 2015).

NS4B engages with NS3 and exerts an influence on viral propagation. It has a dual effect of destabilising NS3 and increasing its helicase activity. The connection between NS4A and the virus is crucial for replicating the virus (Zou et al., 2015). Scientists have focused on inhibiting NS4B, and it has been demonstrated that decreasing it hampers the generation of viral RNA (Van Cleef et al., 2013). In addition, the Novartis research team assessed the effectiveness of spiropyrazolopyridone as medicines that target the NS4B protein. The inhibitors showed efficacy against DENV serotypes 2 and 3 but were ineffective against DENV serotypes 1 and 4 (Zou et al., 2015).

NON-STRUCTURAL PROTEIN-5

The Non-structural protein-5 (NS5) is the most considerable protein in the Dengue Virus (DENV). It has a molecular weight of "104 kDa" and is highly conserved among different strains of DENV. The protein is made up of three functional domains: an "N-terminal MTase" (amino acids 1–296), two "nuclear localisation sequences" (NLS; amino acids 320–405), and an "RdRP domain" (amino acids 273–900) (Yap et al., 2007).

NS5 is cleaved at the "NS4B-NS5 junction" by NS2B and NS3 proteins during the polyprotein production (Cahou et al., 1992). Protein kinase G phosphorylates it, and it possesses two crucial cavities, namely cavities A and B, that are necessary for its function (Zou et al., 2011). The F1 motif located in the NS5 F region plays a crucial role in viral replication by controlling the synthesis of RNA through the promoter (Iglesias et al., 2011). NS5 is crucial for viral replication because it inhibits RNA replication through its RdRp activity. Recombinant NS5, a protein preserved in DENV, has been shown to induce immunogenic responses in BALB/c mice when utilised as an antigen (Alves et al., 2016). These data indicate that NS5 has the potential as an antigen to be included in DENV vaccine formulations.

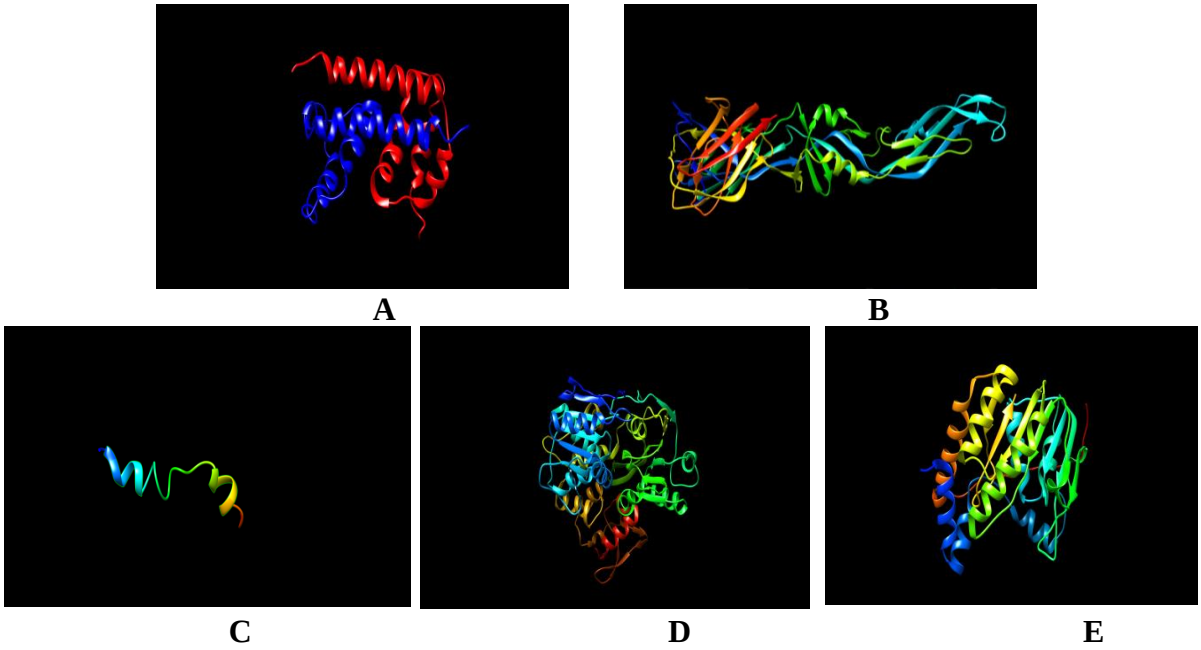

Figure 2: Some structural and non-structural dengue virus proteins and representative PDB structures and IDs. A - Capsid protein (PDB ID 1R6R). B - Envelope protein (PDB ID 1OK8). C - NS2A protein (PDB ID 2M0S). D - NS3 protein (PDB ID 2JLQ). E - NS5 protein (PDB ID 5IKM).

DENGVAXIA - A FAINT HOPE

Dengvaxia, created by Sanofi Pasteur, is the sole authorised vaccine for the infection caused by the dengue virus. This vaccine is approved for individuals between the ages of 9 and 16 who have had dengue in the past (Prompetchara et al., 2019). Dengvaxia is a vaccine that stimulates the production of "prM and E" proteins from all four dengue virus serotypes. It uses a yellow fever virus backbone vector (Swaminathan & Khanna N., 2019). After obtaining marketing permission in 2015, Dengvaxia has become accessible in various Asian and Latin American nations.

Dengvaxia has shown various levels of success in clinical tests undertaken in Southeast Asia, Thailand, and Latin America, with reported rates of 56.5 percent, 30.2 percent, and 60.8 percent, respectively. Nevertheless, studies have indicated that Dengvaxia has reduced efficacy in children below the age of nine and in adults who have not previously contracted the dengue virus (Swaminathan & Khanna N., 2019).

Aside from Dengvaxia, two more tetravalent dengue vaccines are being tested for their effectiveness in Phase III trials. One of these vaccines is being developed by the National Institute of Allergy and Infectious Diseases (NIAID) in partnership with the Butantan Institute, while Takeda Pharmaceuticals is creating the other. Although there have been significant breakthroughs, no vaccine has proven effective against all serotypes of the dengue virus in all age groups. Therefore, additional investigation into the dengue virus is essential to design vaccines or therapies that can efficiently control infection.

PROSPECTS FOR DEVELOPING AN EFFECTIVE ANTI-DENGUE TREATMENT

The increasing worldwide occurrence of dengue highlights its significant influence on public health. The dengue virus relies on crucial structural proteins (E, prM, and C) to coordinate important viral activities like fusion, entrance, assembly, and secretion. On the other hand, NS proteins (NS3, NS2B3, NS5, NS2A, NS4A, and NS4B) aid in viral replication and regulate the immunological responses of the host. Unravelling the complex functions of these proteins shows potential for creating potent antiviral medications or vaccines.

An essential goal in the treatment of dengue is to prevent the virus from entering the host's

cells. Vaccines are considered highly promising for achieving this objective. Nevertheless, developing vaccines has challenges like cross-reactivity and Antibody-Dependent Enhancement (ADE). The E-protein, which plays a crucial role in viral entry, is identified as a critical target for vaccines. However, the differences across the four dengue serotypes make it challenging to develop a universal vaccine formulation. Research suggests that vaccinations based on D-I/D-II have the potential to generate a response that can interact with other flaviviruses. However, D-III vaccines produce antibodies that neutralise the virus without causing considerable antibody-dependent enhancement (ADE). However, the absence of recognised cellular receptors for D-III presents difficulties. In addition, whereas prM protein antibodies have shown effectiveness in protecting mice, they may increase the risk of infection in people due to antibody-dependent enhancement (ADE).

By focusing efforts on the capsid protein, it is possible to avoid cross-reactivity and improve the effectiveness of vaccine development. The combination of capsid and D-III epitopes shows potential for producing efficient vaccinations. Despite the ongoing challenges of toxic side effects and viral resistance, NS3 and NS5 proteins are identified as key targets in therapeutic research. Balapiravir, a medication initially developed for hepatitis C, suppresses NS5 but has slight effectiveness against dengue. Potential treatment approaches can be explored by targeting the interaction between prM and the envelope protein and host components such as cytoskeletons or viral NS proteases. RNA-based techniques, including RNA silencing and peptide-based methods, also show potential. Peptides require increased stability to prevent virus attachment or hinder reproduction (Bray M, Lai CJ. 1991; Luo D, Vasudevan SG, et al., 2015; Martínez MA. 2010).

CONCLUSION

Dengue fever is a significant global issue, with around 390 million cases annually. Despite much scientific research, an effective therapy for it remains elusive. Gaining a comprehensive comprehension of the mechanisms of the dengue virus and the proteins it produces will facilitate the discovery of more effective strategies to manage the disease. The sole authorised vaccination, Dengvaxia, is currently associated with specific issues and concerns therefore improved pharmaceuticals for dengue are urgently required.

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