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REVIEW ARTICLE

Dengue Viruses at a Glance

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ABSTRACT

Dengue is an important global threat caused by dengue virus (DENV) that records an estimated 390 million infections per year. There is therefore an urgent need for the development of antiviral drugs for the treatment of dengue. Peptide molecules of some important proteins are a good choice of medical treatment. In this review, the discovery, structure, serotype, replication and infectious cycle of dengue virus are discussed. The development of peptides acts as the inhibitor molecule for virus entry, replication and translation is also described, with a focus on the three main targets, namely, the host cell receptors, viral structural proteins and viral non-structural proteins. The antiviral peptides designed based on these approaches may lead to the discovery of novel anti- DENV therapeutics that can treat dengue patients.

Keywords: Dengue virus, Drug discovery, Peptides, Antiviral therapeutics. RNA virus.

1. INTRODUCTION

Dengue virus (DENV) infection is the most common mosquito-transmitted viral infection. DENV infection can cause mild dengue fever or severe dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). Hemorrhage and vascular leakage are two characteristic symptoms of DHF/DSS. However, due to the limited understanding of dengue pathogenesis, no satisfactory therapies to treat nor vaccine to prevent dengue infection are available, and the mortality of DHF/DSS is still high. Viruses are tiny agents that can infect a variety of living organisms, including bacteria, plants, and animals. Like other viruses, the dengue virus is a microscopic structure that can only replicate inside a host organism.

1.1 Discovery of Dengue Viruses

In 1943, Ren Kimura and Susumu Hotta first isolated the dengue virus. These two scientists were studying blood samples of patients taken during the 1943 dengue epidemic in Nagasaki, Japan. A year later, Albert B. Sabin and Walter Schlesinger independently isolated the dengue virus. Both pairs of scientists had

isolated the virus now referred to as dengue virus 1 (DENV1). The dengue viruses are members of the *Flavivirus* genus of the *Flaviviridae* family. The Dengue infection usually shows severe head ache, fever, rashes all over the body and finally leads to death. Dengue hemorrhagic fever has high mortality rate. Dengue virus (DENV) is the most significant arthropod-borne viral pathogen in humans. Along with the dengue virus, this genus also includes a number of other viruses transmitted by mosquitoes and ticks that are responsible for human diseases. The *Flavivirus* genus includes other important emerging and reemerging human pathogens such as Zika virus (ZIKV), West Nile virus (WNV), Japanese encephalitis virus (JEV), Yellow fever virus (YFV), and Saint Louis encephalitis virus (SLEV). Most flaviviruses are arthropod-borne; however, vertebrate-and invertebrate-specific viruses are also members of the group. DENV cycles in nature between *Aedes* mosquito vectors (mainly *Aedes albopictus* and *Aedes aegypti*) and humans. Four DENV serotypes (DENV1, DENV2, DENV3, and DENV4) circulate in tropical and subtropical regions of the globe.

1.2 Dengue Serotypes

Dengue infections are caused by four closely related viruses named (DENV1, DENV2, DENV3, and DENV4). These four viruses are called serotypes because each has different interactions with the antibodies in human blood serum. The four dengue viruses are similar — they share approximately 65% of their genomes — but even within a single serotype, there is some genetic variation. Despite these variations, infection with each of the dengue serotypes results in the same disease and range of clinical symptoms. Are these four viruses all found in the same regions of the world? In the 1970s, both DEN-1 and DEN-2 were found in Central America and Africa, and all four serotypes were present in Southeast Asia. DENV nonstructural protein 1 (NS1), which can be secreted in patient's sera, has been used as an early diagnostic marker for dengue infection for many years. However, the roles of NS1 in dengue-induced vascular leakage were described only recently. The pathogenic roles of DENV NS1 in hemorrhage and vascular leakage are reviewed, and the possibility of using NS1 as a therapeutic target and vaccine candidate is discussed. By 2004, however, the geographical distribution of the four serotypes had spread widely. Now all four dengue serotypes circulate together in tropical and subtropical regions around the world. The four dengue serotypes share the same geographic and ecological niche.

After suffering from an infection with one dengue serotype, a person develops an immunity against that particular serotype. Individuals are protected from infections with the remaining three serotypes for two to three months after the first dengue infection. However, it is not lifelong protection. After that short period, a person can be infected with any of the remaining three dengue serotypes. Researchers have noticed that subsequent infections can put individuals at a greater risk for severe dengue illnesses than those who have not been previously infected.

1.3 General information about Dengue Viruses

Dengue virus (DENV) is the most common mosquito-borne flavivirus and threatens people in tropic and subtropical areas. The World Health Organization estimates that more than 3.0 billion people representing over 40% of the world's population are at risk of dengue infection. DENV is estimated to cause around 390 million infections occur annually, of which 96 million manifest clinically, placing over 3 billion people at risk of infection [1]. Dengue virus infections are often asymptomatic or cause a flu-like syndrome with fever and rash. However, a small proportion of cases develop into severe illness, which is termed dengue hemorrhagic fever (DHF).

DHF is characterized by vascular leakage, thrombocytopenia, and coagulopathy [2]. Vascular leakage results in hemoconcentration and serous effusions, leading to circulatory collapse, which further develops into life-threatening dengue shock syndrome (DSS) [2]. An estimated 390 million infections occur each year globally, and approximately 960,000 people with severe dengue require hospitalization [1]. Children contribute to a large proportion of the severe disease cases. In 1958, DHF was reported to carry a case fatality rate (CFR) of 13.9% in Bangkok [3]. Even with standardized diagnosis and management, the CFR remained in the range of 0.5–1.7% from 2000–2011 in the Philippines [4]. Despite the high mortality of DHF/DSS, no promising viral-specific drugs or vaccines are available due to the limited understanding of the complicated pathogenic mechanism.

Several hypotheses have been proposed to explain the pathogenesis of DHF/DSS [5]. Among them, antibody-dependent enhancement (ADE) has been proposed to explain why most DHF/DSS cases occur in children who are secondarily infected with a different serotype of DENV from the previous one [6]. Based on ADE, antibodies that are generated by a single DENV infection contribute to lasting homotypic immunity but may permit heterotypic DENV infection. Furthermore, these serotype non-specific antibodies may augment heterotypic virus entry and replication in Fcγ receptor-bearing macrophages, leading to enhanced viremia, antigenemia and cytokine storm [7]. This scenario may also explain why infants who passively acquire maternal anti-dengue antibodies are more likely to develop DHF/DSS following primary infection [8]. However, ADE dose not explain why vascular leakage and hemorrhage occur in DHF/DSS patients. Only when we better understand the molecular mechanisms of DENV pathogenesis can a more effective and specific therapy or vaccine against DHF/DSS be developed. In this review, we focus on the pathogenic roles of DENV non-structural protein 1 (NS1) in the pathogenesis of DHF/DSS. The potential of NS1 as a drug target or vaccine candidate to treat or prevent dengue will be discussed.

1.4 DENV structure

The DENV particle is approximately 500 Å in diameter and includes a positive-sense RNA genome with ~10,700 nucleotides and 3 structural proteins: capsid (C, 100 amino acids), precursor membrane (prM, 75 amino acids), and envelope (E, 495 amino acids) [9].

The capsid protein and a single strand of viral RNA as its genome form a nucleocapsid that buds at the endoplasmic reticulum (ER) in association with 180 copies of prM and E and carries host-derived lipids to form the immature virion [10].

Initially, the immature virion is covered by 60 spikes, each of which is composed of E trimers with associated prM proteins. The maturation process requires the host protease furin, which cleaves prM into the pr and M proteins in the Golgi after the noninfectious virion passes through the cell's secretory system, which is an acidic environment. This cleavage results in a rearrangement of E to the immature dimer structure, in which E maintains interactions with pr and M [11].

After budding from the cell via exocytosis, the neutral pH of the extracellular environment dissociates E and pr to form mature virions, which are available to infect new cells [11]. In addition to the structural proteins, the RNA genome of dengue virus encodes 7 nonstructural proteins that are essential for viral replication (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5).

Recently, the pathogenic roles for secreted NS1 in DHF/DSS have been demonstrated due to its involvement in systemic immunity and endothelial cell activation. In this review, we focus on the molecular mechanisms underlying how NS1 may contribute to vascular leakage, coagulopathy and thrombocytopenia during dengue infection. The possibility of targeting NS1 as a drug and vaccine development target against dengue infection will also be discussed.

1.5 Dengue Virus Genome and Structure

The dengue virus genome is a single strand of RNA. It is referred to as *positive-sense RNA* because it can be directly translated into proteins. The viral genome encodes ten genes . The genome is translated as a single, long polypeptide and then cut into ten proteins.The dengue virus genome encodes three structural proteins: (capsid [C], membrane [M], and envelope [E]) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins.

Dengue virus is a small virus that carries a single strand of RNA as its genome. The genome encodes only ten proteins. Three of these are structural proteins that form the coat of the virus and deliver the RNA to target cells, and seven of them are nonstructural proteins that orchestrate the production of new viruses once the virus gets inside the cell. The outermost structural protein, termed the envelope protein, the virus is enveloped with a lipid membrane, and 180 identical copies of the envelope protein are attached to the surface of the membrane by a short transmembrane segment. The job of the envelope protein is to attach to a cell surface and begin the process of infection.

Three are structural proteins: the capsid (C), envelope (E), and membrane (M) proteins. Seven are nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. These nonstructural proteins play roles in viral replication and assembly.The structure of the dengue virus has a roughly spherical shape. Inside the virus is the nucleocapsid, which is made of the viral genome and C proteins. The nucleocapsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken from the host. Embedded in the viral envelope are E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells. The structure of the dengue virus is roughly spherical, with a diameter of approximately 50 nm (1 nm is one millionth of 1 mm) (Figure 3). The core of the virus is the nucleo capsid, a structure that is made of the viral genome along with C proteins. The nucleo capsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken from the host. Embedded in the viral envelope are 180 copies of the E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells.

1.6 Dengue Virus Replication and Infectious Cycle

The dengue viral replication process begins when the virus attaches to a human skin cell (Figure 4). After this attachment, the skin cell's membrane folds around the virus and forms a pouch that seals around the virus particle. This pouch is called an endosome. A cell normally uses endosomes to take in large molecules and particles from outside the cell for nourishment. By hijacking this normal cell process, the dengue virus is able to enter a host cell.

The dengue virus attaches to the surface of a host cell and enters the cell by a process called endocytosis. Once deep inside the cell, the virus fuses with the endosomal membrane and is released into the cytoplasm. The virus particle comes apart, releasing the viral genome. The viral RNA (vRNA) is translated into a single polypeptide that is cut into ten proteins, and the viral genome is replicated. Virus assembly occurs on the

surface of the endoplasmic reticulum (ER) when the structural proteins and newly synthesized RNA bud out from the ER. The immature viral particles are transported through the trans-Golgi network (TGN), where they mature and convert to their infectious form. The mature viruses are then released from the cell and can go on to infect other cells.

Once the virus has entered a host cell, the virus penetrates deeper into the cell while still inside the endosome. How does the virus exit the endosome, and why? Researchers have learned that two conditions are needed for the dengue virus to exit the endosome:

1. The endosome must be deep inside the cell where the environment is acidic.
2. The endosomal membrane must gain a negative charge.

These two conditions allow the virus envelope to fuse with the endosomal membrane, and that process releases the dengue nucleocapsid into the cytoplasm of the cell.

Once it is released into the cell cytoplasm, how does the virus replicate itself? In the cytoplasm, the nucleocapsid opens to uncoat the viral genome. This process releases the viral RNA into the cytoplasm. The viral RNA then hijacks the host cell's machinery to replicate itself. The virus uses ribosomes on the host's rough endoplasmic reticulum (ER) to translate the viral RNA and produce the viral polypeptide. This polypeptide is then cut to form the ten dengue proteins.

The newly synthesized viral RNA is enclosed in the C proteins, forming a nucleocapid. The nucleocapsid enters the rough ER and is enveloped in the ER membrane and surrounded by the M and E proteins. This step adds the viral envelope and protective outer layer. The immature viruses travel through the Golgi apparatus complex, where the viruses mature and convert into their infectious form. The mature dengue viruses are then released from the cell and can go on to infect other cells.

2. SUMMARY

The dengue virus is a tiny structure that can only replicate inside a host organism. The four closely related dengue viruses — DEN-1, DEN-2, DEN-3, and DEN-4 — are found in the same regions of the world. The dengue virus is a roughly spherical structure composed of the viral genome and capsid proteins surrounded by an envelope and a shell of proteins. After infecting a host cell, the dengue virus hijacks the host cell's machinery to replicate the viral RNA genome and viral proteins. After maturing, the newly synthesized dengue viruses are released and go on to infect other host cells. In this paper, we discuss recent advances in our knowledge of the dengue virus life cycle based on new structural data of the virus and its proteins. These structures provide a basis for describing function and predicting putative host interactions.

3. ABBREVIATIONS

ADE: antibody-dependent enhancement**, CFR:** Case-fatality rate**, CXCR:** CXC chemokine receptors, **DENV:** Dengue virus **DF:** Dengue fever, **DHF:** Dengue hemorrhagic fever, **DSS:** Dengue shock syndrome, **E:** Envelope protein, **ER:** Endoplasmic reticulum, **GAG:** Glycosaminoglycan, **SCF:** soluble complement-fixing**.**

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