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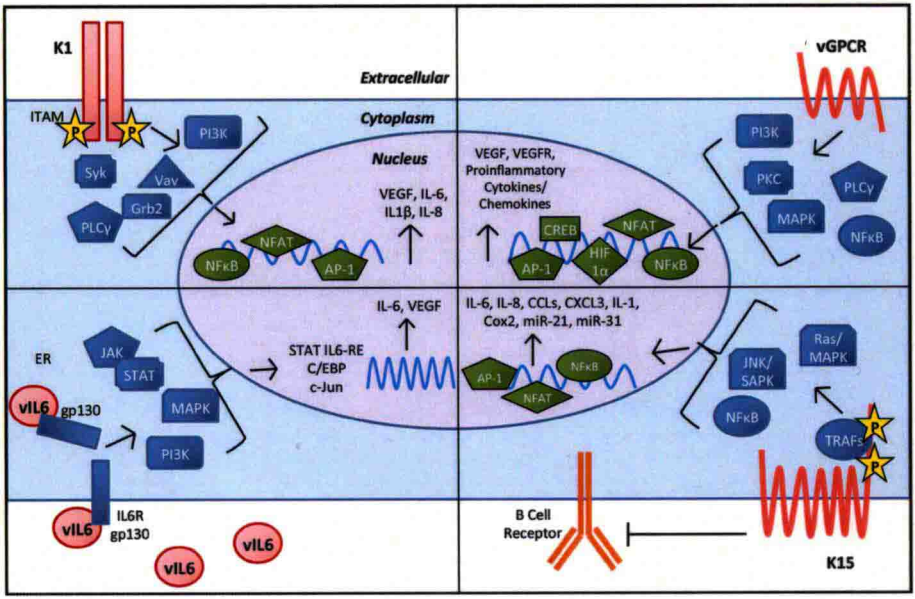

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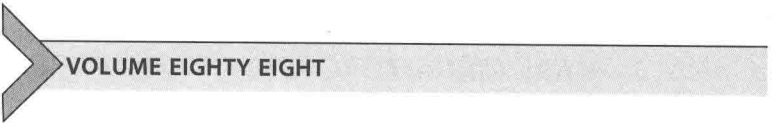

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CHAPTER ONE

Revisiting Dengue Virus–Host Cell Interaction: New Insights into Molecular and Cellular Virology

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Abstract

Dengue virus (DENV) is an emerging mosquito-borne human pathogen that affects millions of individuals each year by causing severe and potentially fatal syndromes. Despite intense research efforts, no approved vaccine or antiviral therapy is yet available. Overcoming this limitation requires detailed understanding of the intimate relationship between the virus and its host cell, providing the basis to devise optimal prophylactic and therapeutic treatment options. With the advent of novel high-throughput technologies including functional genomics, transcriptomics, proteomics, and lipidomics, new important insights into the DENV replication cycle and the interaction of this virus with its host cell have been obtained. In this chapter, we provide a comprehensive overview on the current status of the DENV research field, covering every step of the viral replication cycle with a particular focus on virus–host cell interaction. We will also review specific chemical inhibitors targeting cellular factors and processes of relevance for the DENV replication cycle and their possible exploitation for the development of next generation antivirals.

1. INTRODUCTION

Dengue is the most prevalent mosquito-borne viral disease affecting humans. During the past four decades, dengue fever (DF) has emerged as a global public health problem, with outbreaks reported in more than 100 countries in the Asia–Pacific, the Americas, the Middle East, and Africa. Several demographic and societal changes including rapid and unplanned urbanization, globalization, and increased international travel have contributed to the geographic expansion of the main mosquito vector *Aedes aegypti* and consequently to the increase in the incidence of dengue virus (DENV) infections. It is estimated that around 3.6 billion people, more than half of the world's population, live in areas that are at risk for DENV infection (Wilder-Smith et al., 2012). The World Health Organization (WHO) estimates that 50–100 million infections occur each year, although recent estimates indicate that the total number of infections (apparent and in apparent) likely reach 390 million per year (Bhatt et al., 2013).

DENV belongs to the family *Flaviviridae*, which includes several other important human pathogens such as West Nile virus (WNV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and hepatitis C virus (HCV). Infection of humans with any of the four antigenically distinct DENV serotypes (DENV-1 to DENV-4) can result in a broad spectrum of clinical outcomes including the two well-defined syndromes, DF and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), and a range of intermediate responses or no clinical response at all.

In spite of the great progress made during the past 50 years in the dengue research field, dengue remains a disease with many unanswered questions and no vaccine or antiviral therapy is currently available. Understanding the basic biology of DENV and its interaction with the host cell may help to design efficient strategies for the control of the disease.

2. DENGUE DISEASE

2.1. History and epidemiology

The earliest reports of illness resembling DF were recorded in a Chinese medical encyclopedia dated 992 (Gubler, 2006). However, the first epidemics of well-documented cases of what is believed to be dengue occurred in 1779–1780 in Asia, Africa, and North America, suggesting that the insect vector was widespread already prior to the eighteenth century (Guzman & Isturiz, 2010). The viral etiology and mosquito transmission of dengue were demonstrated in 1907 in a series of experiments in which mosquitoes fed on patients with acute dengue were used to infect healthy volunteers from the US Army (Ashburn, Craig, & US Army Board for the Study of Tropical Diseases, 2004), but it was not until World War II that the first two serotypes were isolated, followed by the isolation of the two remaining serotypes in 1954 (Hammon, Rudnick, & Sather, 1960; Hotta, 1952; Sabin & Schlesinger, 1945).

Significant ecological and demographic changes caused by World War II facilitated the spread of both the mosquito vector and the virus in the Asia-Pacific region (Gubler, 2006; Kuno, 2007). Uncontrolled urbanization, inadequate waste, and sewer management as well as lack of vector control programs eventually resulted in a tremendous increase in epidemic activity and the emergence of DHF. The first epidemic of DHF was recorded in the Philippines in 1953–1954, which was followed by another epidemic in 1958. Since then, all four serotypes of DENV are circulating in this region (Guzman & Isturiz, 2010). Nowadays, the Asian countries with the highest

number of dengue cases are the Philippines, Thailand, and Vietnam. China represents a particular case. Twenty percent of the world's population, that is around 1.3 billion people, lives in this country and various outbreaks have been reported in the 1980s and 1990s. However, since 2003, the WHO has not received any other reports of dengue, making the real situation in this country impossible to determine (Guzman & Isturiz, 2010). Likewise, insufficient epidemiological surveillance data are available for Africa. Nevertheless, some reports indicate that outbreaks of the four serotypes have increased dramatically since 1980 (Guzman & Isturiz, 2010).

The current epidemiological situation of dengue in the Americas is also alarming. A 4.6-fold increase in the total reported cases has been registered consistently during the last three decades, with a worrisome 8.3-fold increase in the number of cases of DHF (San Martin et al., 2010). During the 1980s, the highest number of cases was found in the Hispanic Caribbean, but there was a shift to the Southern Cone starting from the early 1990s (San Martin et al., 2010). Recent outbreaks in this region include large and densely populated areas such as Rio de Janeiro (Brazil), with registered epidemics in 2002 and 2008, and Bolivia in early 2009. In the same year, the disease spread as far as Buenos Aires (Argentina) (Guzman & Isturiz, 2010; Seijo, 2009).

In Europe, many cases of imported DF in returning travelers are registered each year (Jelinek et al., 2002). However, in 2010, the first cases of autochthonous infections were reported in Croatia and France, presumably transmitted by *Aedes albopictus*, a secondary vector of DENV whose eggs are somewhat resistant to subfreezing temperatures (Gjenero-Margan et al., 2011; Gould, Gallian, De Lamballerie, & Charrel, 2010; La Ruche et al., 2010).

2.2. Transmission and course of infection

DENV is transmitted to humans by the bite of infected mosquitoes of the genus *Aedes*. During their blood meal, infected mosquitoes inject the virus subcutaneously, where it encounters several cells of the immune system. The cell types that are first infected have not been extensively studied, but it is thought that skin-resident macrophages and dendritic cells (DCs) are initial targets (St John, Abraham, & Gubler, 2013). Infected cells subsequently migrate to lymph nodes, where recruited macrophages and monocytes become additional targets, thus amplifying the infection further (Martina, Koraka, & Osterhaus, 2009). As dissemination progresses, the virus is detected in draining and remote lymph nodes, finally resulting in viremia (Marchette, Halstead, Falkler, Stenhouse, & Nash, 1973).

Although DCs, monocytes, and macrophages are considered as the major sites of virus replication in humans (Jessie, Fong, Devi, Lam, & Wong, 2004; Limon-Flores et al., 2005; Wu et al., 2000), the virus could also be detected in various other tissues, including spleen, kidneys, lungs, and the liver (Jessie et al., 2004; Seneviratne, Malavige, & de Silva, 2006). Infected endothelial cells have been detected in human infections, but the importance of this replication site is discussed controversially (Balsitis et al., 2009; St John et al., 2013).

Viremia can be detected 24–48 h before the onset of clinical symptoms and lasts up to 10 days. Mosquitoes feeding on viremic individuals take up the virus, which then infects epithelial cells of the midgut. The virus is subsequently disseminated into the hemocoel and finally reaches the salivary glands (Salazar, Richardson, Sanchez-Vargas, Olson, & Beaty, 2007). After an incubation period of 4–12 days postfeeding (Salazar et al., 2007), mosquitoes become infectious and are able to transmit DENV.

The most commonly reported symptomatic outcome of DENV infection is DF characterized by a sudden onset of fever and a variety of nonspecific symptoms including headache, myalgia, body aches, retro-orbital pain, rashes, and joint pains (Whitehorn & Simmons, 2011). The disease is generally self-limiting with the acute phase lasting up to 1 week followed by a convalescent phase extending to several weeks. Some estimates indicate that between 1% and 70% of patients suffering from DENV infection have mild hemorrhagic manifestations such as petechiae, purpura, ecchymoses, and epistaxis (Aggarwal, Chandra, Aneja, Patwari, & Dutta, 1998; Vaughn et al., 2000). In up to 2% of the cases (mostly in children under the age of 15) (Gubler, 1998), the disease may progress to a more severe and life-threatening DHF, characterized by liver damage, increased vascular permeability, thrombocytopenia, and hemorrhagic manifestations at the skin, nose, gum, and gastrointestinal tract (Halstead, 2007; Kyle & Harris, 2008). DSS, the most severe form of DHF, is characterized by weak pulse and sudden drop in blood pressure, which is the result of collapse of the vascular system owing to hypovolemia caused by vascular leakage (St John et al., 2013).

2.3. Pathogenesis

It is not fully understood why most patients resolve DENV infections quickly and without complications, whereas others experience a potentially fatal vascular leak syndrome or severe hemorrhages. Attempts to

explain the pathogenesis of dengue in all its complexity must consider clinical, immunologic, pathological, and epidemiological features of DENV infection. Comprehensive reviews of this topic have recently been published (Costa, Fagundes, Souza, & Teixeira, 2013; Martina et al., 2009; Whitehorn & Simmons, 2011). In this section, we summarize only some of the predominant theories that might explain DHF/DSS pathogenesis.

Primary DENV infection confers long-lasting immunity to the infecting serotype and partial immunity to subsequent infection with other serotypes. Epidemiological observations show that secondary infection with a heterologous serotype is a risk factor to develop severe forms of the disease. One theory assumes that the higher incidence of DHF/DSS upon secondary infections is due to antibody-dependent enhancement (ADE) (Halstead, 2007). Thus, antibodies from a primary infection are cross-reactive with other DENV serotypes, but do not neutralize the infection. These antibodies could then mediate increased uptake of opsonized virus particles into Fcγ receptor-bearing cells (i.e., DCs, monocytes, and macrophages), resulting in increased viral replication and immune activation accompanied by enhanced cytokine release (Halstead, 2007).

An analogous mechanism might operate at the level of activated T cells, designated "original antigenic sin" (Mongkolsapaya et al., 2003). This model argues for a reactivation of cross-reactive memory T cells specific for the primary DENV infection inducing increased cytokine secretion, higher virus titers due to delayed viral clearance, and apoptosis of both infected and uninfected bystander cells (Mongkolsapaya et al., 2003).

In both models, cytokines play a direct role in immunopathogenesis of dengue, resulting from their proinflammatory effects on vascular endothelial cells that would lead to leaky junctions and increased capillary permeability (Pang, Cardosa, & Guzman, 2007). In fact, elevated levels of numerous cytokines have been observed during the course of DENV infection. In particular, high concentrations of IFN-γ, TNF-α, and IL-10 have been detected in the sera of infected patients (Chakravarti & Kumaria, 2006; Nguyen et al., 2005; Perez et al., 2004), and elevated levels of IL-6 were detected in children with DSS (Juffrie et al., 2001). However, these hypotheses cannot explain severe courses of disease after primary DENV infections. Thus, several additional factors such as the activation of the complement system, virus virulence, and, most importantly, host genetic factors may account for DENV pathogenesis (Costa et al., 2013; Martina et al., 2009; Whitehorn & Simmons, 2011).

3. THE MOLECULAR BIOLOGY OF DENV

3.1. An overview of the DENV replication cycle

The infection process starts with the attachment of the incoming virions onto the surface of susceptible cells (Fig. 1.1). The viral envelope protein is reported to bind to a diverse array of low- and high-affinity cellular surface receptors allowing cell entry by receptor-mediated endocytosis. The acidification of late endosomes triggers the fusion of the viral envelope with the endosome membrane, thus releasing the nucleocapsid into the cytoplasm. The nucleocapsid dissociates and the released RNA is translated by ribosomes at membranes of the rough ER. The resulting polyprotein is cleaved by host and viral proteases into three structural proteins (C, prM, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The nonstructural proteins, presumably together with host proteins, induce membrane invaginations into the ER lumen that are linked to the cytoplasm via 11 nm pore-like openings (Welsch et al., 2009) (Fig. 1.1). It is assumed that the interior of the vesicle harbors the viral replicase that is responsible for genome amplification. This is mediated primarily by the NS5 RNA-dependent RNA polymerase (RdRP) that generates a complementary negative strand, which is used as template to generate multiple copies of genomic RNA. These contain a type 1 cap at the 5′-end, which is generated by the methyltransferase (MTase) and the guanylyltransferase (GTase) activities of NS5, in conjunction with the RNA 5′-triphosphatase (RTPase) activity of NS3. Newly generated viral RNAs associate with capsid proteins to form nucleocapsids that bud into the lumen of the ER at prM- and E-rich microdomains. The resulting immature virions are transported from the ER to the extracellular milieu via the conventional secretory pathway (Fig. 1.1). While passing through the trans-Golgi network (TGN), the host protease furin cleaves the prM protein on the virion surface into the M-peptide that remains associated with the virion and the pr peptide that is released, thus giving rise to mature infectious virus particles.

3.2. The Dengue virus particle: Biogenesis, release, and entry properties

The DENV particle consists of an inner nucleocapsid composed of a single copy of genomic RNA associated with ~180 copies of the capsid protein (molecular weight ~12 kDa) (Zhang, Kostyuchenko, & Rossmann, 2007). The nucleocapsid is surrounded by a lipid membrane bilayer