
Dengue Virus: Past, Present and Future

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Abstract

Dengue is an old disease caused by the mosquito-borne dengue viruses (DENV-1–4). In the last century, dengue has escalated in geographic distribution and disease severity to become now the most common arboviral infection of humans in the subtropical and subtropical regions of the world. In this chapter the authors discuss the historical aspects of dengue virus transmission and factors contributing to its evolution as one of the most important public health problems of this century.

Dengue fever – an old disease spreading with a new vengeance

Across much of the world, people bitten by an *Aedes aegypti* mosquito may soon find themselves prostrated by the high fever and severe joint pain that are the classic symptoms of dengue fever, and to some extent this is nothing new; reports of symptoms consistent with dengue date back over two millennia (Gubler, 2006). Why then is a book about frontiers in dengue virus needed at this time? The reason: although dengue is an old disease, recent decades have seen an unprecedented increase in the geographic range, incidence, and severity of dengue infection (Gubler, 2006; Kyle and Harris, 2008).

Dengue disease is caused by the four serotypes of mosquito-borne dengue virus (DENV-1–4), positive-sense RNA viruses belonging to the genus *Flavivirus*. Escalation of the dengue pandemic can largely be attributed to three factors: (i) increased urbanization and consequent

urban detritus and population density leading to enhanced vector breeding and increased contact between humans and vectors, (ii) global invasion of the major mosquito vectors, *Aedes aegypti* and *Aedes albopictus*, leading to geographic spread and geographic overlap of all four dengue virus serotypes and (iii) interaction and evolution of the four serotypes themselves, resulting in greater disease severity (Gubler, 2006; Kyle and Harris, 2008). As a result of these changes, DENV is now the most common arboviral infection of humans in the subtropical and subtropical regions of the world. The World Health Organization (WHO) estimates that 2.5 billion people are at risk from dengue with 50 million dengue infections worldwide every year (Anonymous, 2008). In 2007, there were more than 890,000 reported cases of dengue in the Americas, approximately 26,000 of which were the most severe form, dengue haemorrhagic fever (DHF). The WHO reports that dengue disease is endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific, with South-East Asia and the Western Pacific the most seriously affected. Approximately 500,000 people with DHF require hospitalization each year, of whom 2.5% die.

Scope and direction of frontiers in dengue virus research

It is our hope that this book will provide a foundation for the response to the public health emergency posed by dengue virus. We have made an effort not just to review the rapidly expanding

dengue research literature, but also to identify the most pressing questions that remain to be answered about dengue biology and control. The remainder of this chapter provides an overview of the evolutionary history and epidemiology of dengue virus. The chapters in section two cover translation and processing of the dengue virus polyprotein, viral replication, and the role of the viral untranslated regions in regulation of genome synthesis and translation. Section three presents current knowledge on the pathogenesis of and host immune response to dengue illness, focusing on the role of host and virus determinants of susceptibility and dengue disease severity, changes in protein expression in infected hosts, virus modulation of the host immune response, and development of animal models in which to study dengue virus pathogenesis. Section four discusses the crucial topic of the epidemiology and evolutionary dynamics of DENV, with chapters on DENV–mosquito interactions, evolutionary dynamics of dengue virus, temporal and spatial dynamics of dengue virus transmission, and emergence of DENV from its ancestral, sylvatic cycle. Finally, section five addresses various approaches that are currently being developed in the control of dengue disease, including vaccines, novel drugs, and passive immunotherapy.

Evolution of the flaviviruses

It is not known with certainty when and where the progenitor of the approximately 80 species in the genus *Flavivirus* first arose, although geographic evidence suggests that this ancestral flavivirus may have first appeared in Africa. Over the course of speciation, the flaviviruses have shown substantial ecological diversification. Most notably, different lineages of flaviviruses adapted to different modes of transmission. A current phylogenetic tree of the genus *Flavivirus* (Fig. 1.1) shows that the basal-most lineages are viruses that have only been isolated from mosquitoes and are not known to infect vertebrates at all. This suggests that the ancestor of the genus may have been a ‘mosquito-only’ virus that later acquired the ability to infect vertebrates. The remaining flaviviruses are divided into vector-borne viruses of vertebrates, with major groups using ticks and mosquitoes for horizontal transmission, and another group that infects vertebrates without

the use of arthropod vectors. This topology does not suggest whether vector-borne or non-vector-borne transmission was ancestral, but the basal position of the ‘mosquito-only’ viruses suggests that mosquito-borne transmission among vertebrates may have preceded the loss of vector transmission. Tick-borne transmission may have evolved from a mosquito-borne lineage after the lineage that infects only vertebrates arose.

All of the flaviviruses known to be human pathogens are transmitted by vectors, and, with the exception of dengue virus, all are zoonoses (Karabatsos, 1985). The Japanese encephalitis virus (JEV) group, consisting of Japanese encephalitis virus, West Nile virus (WNV) and St Louis encephalitis (SLE), among others, is maintained in a cycle of transmission between passerine birds and *Culex* mosquitoes. Mammalian hosts, including humans, can be infected but are an evolutionary dead-end since the viraemia achieved is too low for subsequent transmission (Endy and Nisalak, 2002). The tick-borne encephalitis group is transmitted among rodents by a tick vector; as with the JEV group humans are a dead-end host (Endy and Nisalak, 2002). Yellow fever virus (YFV) is primarily maintained in a sylvatic cycle involving non-human primates and *Aedes* mosquitoes, but it has shown the capacity to adapt to transmission in urban areas using *Aedes aegypti* mosquitoes and humans as its primary reservoir. Such adaptation results in urban epidemics of yellow fever (Monath, 1988). Though each of the species described above shows intraspecific genetic variation, evolution has not led to the divergence of multiple serotypes, consequently humans that survive infection retain life-long protection against re-infection (Rice, 1996). Despite ecological variation among flavivirus species, the organization of the flavivirus genome is conserved throughout the genus. Each positive-sense, single-stranded RNA genome is approximately 11 kb in length and encodes a single polyprotein that is co- and post-translationally processed into three structural and seven non-structural (NS) proteins (Fig. 1.2) (Rice, 1996). This processing event is described in detail in Chapter 2. The structural proteins consist of capsid (C), membrane (the mature form of the pre-membrane (prM) protein) and envelope (E). As discussed in Chapter 13, the E protein contains

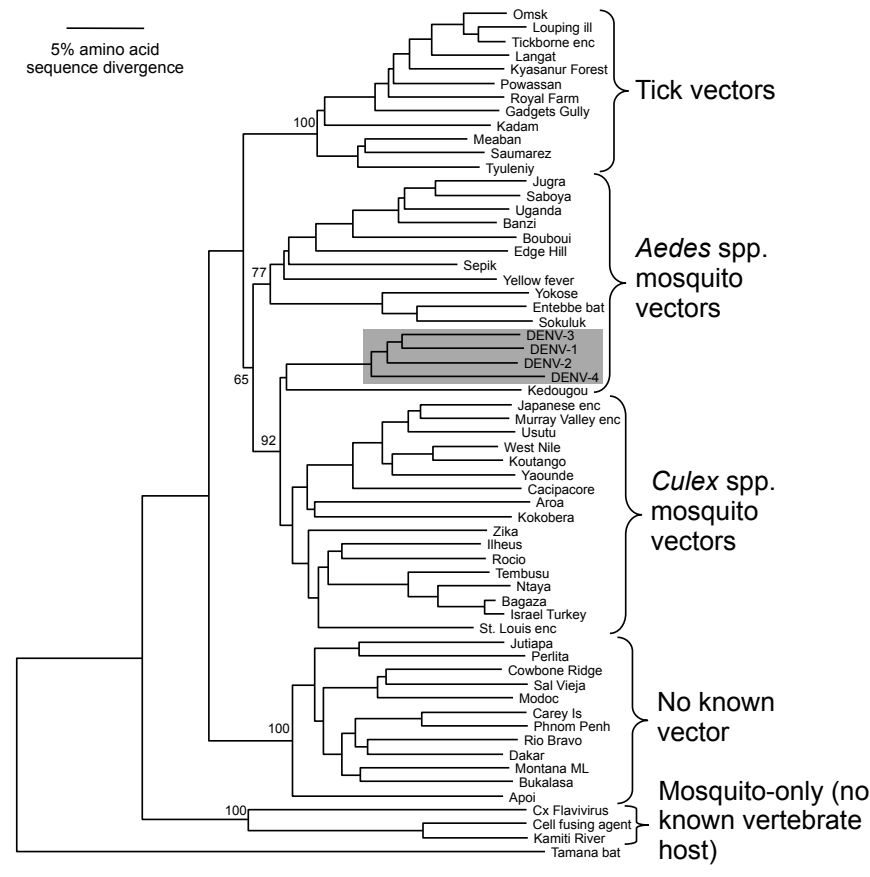


Figure 1.1 Phylogenetic tree is displayed using the neighbour joining method from partial NS5 amino acid sequences available in the GenBank library. Numbers indicate bootstrap values obtained from 1000 replicates for groups to the right. The four serotypes of dengue virus are shaded in grey.

the binding site for the as-yet unidentified cellular receptor; within the endosome E shifts from a homodimer to a homotrimer, enabling fusion with the cell membrane. The functions of some of the flavivirus non-structural proteins have been extensively studied. For example NS5 acts as the RNA-dependent RNA polymerase and possesses a nuclear localization sequence and methyltransferase activity and, as described in Chapter 2, NS2b and NS3 together act as the viral protease. However the function of most non-structural proteins is not well known. Current understanding of the role of each of these NS proteins in the flavivirus replication complex is described in Chapter 3. The genome is flanked at the 5' and 3' termini by untranslated regions whose binding facilitates genome synthesis and whose structure and function are discussed in Chapter 4. The

length of the UTRs varies considerably among different species. The structure of the flavivirus virion, a smooth sphere approximately $\sim 500 \text{ \AA}$ in diameter, was first determined by Kuhn *et al.* (2002) using DENV and is described in further detail in Chapter 13.

Dengue virus evolution

The evolutionary path of dengue virus (discussed in detail in Chapter 9) differs in several important aspects from its flavivirus cousins, though dengue retains many of the same clinical characteristics such as production of severe fever, myalgias, headache, hepatitis, encephalitis and haemorrhage. The phylogeny of the flaviviruses sheds little light on the origin of DENV because the closest relatives include mosquito-borne viruses that occur in several continents (Fig. 1.1). However,



Figure 1.2 The dengue virus genome is displayed demonstrating the coding region flanked at the 5' and 3' termini by untranslated regions (UTRs). The coding region contains three structural genes [capsid (C), pre-membrane (prM), and envelope (E)] as well as seven non-structural (NS) genes. Subdivision of each region represents its relative size; the total genome is approximately 10.6 kb in length.

as described in Chapter 11, more detailed phylogenetic studies of DENV suggest an Asian origin, where sylvatic cycles between non-human primates and *Aedes* mosquitoes arose. Unlike the other flaviviruses however, DENV evolved into four antigenically and phylogenetically distinct serotypes: DENV-1, DENV-2, DENV-3 and DENV-4. Subsequently, each of these four serotypes emerged independently into an endemic cycle of transmission between humans and *Aedes albopictus* (Holmes and Twiddy, 2003). This endemic cycle is now both ecologically and evolutionarily separate from the sylvatic cycle. Thus, unlike other flaviviral pathogens, urban cycles of DENV can no longer be considered zoonotic.

It has been demonstrated that DENV evolves according to a molecular clock at a serotype- and genotype-specific rate (Twiddy *et al.*, 2003a), and that the transfer of DENV from a sylvatic cycle to sustained human transmission may have occurred on the order of 100 to 1500 years ago (Wang *et al.*, 2000), suggesting that the current global pandemic of all four serotypes of DENV appeared during the past century (Twiddy *et al.*, 2003a). The contemporary genetic diversity seen in all four dengue serotypes is related to population growth, urbanization, and mass transport of both virus and its mosquito vector. Using an analytical technique based on coalescent theory, Holmes and Twiddy demonstrated that DENV-2 and DENV-3 experienced two phases of exponential growth (Twiddy *et al.*, 2003b). In the first phase and for most of their history, the dengue viruses experienced a low rate of exponential growth. Thirty years ago, the rate of growth of DENV-2 and DENV-3 suddenly increased by a factor of between 15 and 20.

Dengue virus epidemiology

The spread of *Aedes aegypti* mosquitoes through the slave trade and later through the movement of ships and goods during the Second World War

facilitated the global expansion of dengue virus (see Chapter 8). The first descriptions of dengue fever characterized the eighteenth-century pandemic of dengue infection as described in 1780 by Benjamin Rush during a large outbreak of dengue fever in Philadelphia, Pennsylvania, in the USA (Rush, 1789). Dengue was thought to have been introduced in the USA as a consequence of the rum and slave trade between Africa and Caribbean ports. Dengue outbreaks occurred throughout the USA, the Caribbean and South America during the nineteenth and early twentieth centuries (Halstead, 1992). The second dengue pandemic was centred in the mining towns of northern Queensland, Australia, where boom towns and resulting *Aedes aegypti* population growth resulted in continuous dengue transmission from the 1870s until the First World War (Halstead, 1992). Dengue outbreaks were also occurring in the Eastern Mediterranean and resulted in a large epidemic in Greece during 1928. During the Second World War, dengue strains were carried by ships and soldiers from South-East Asia to Japan, the Pacific Islands, Philippines and Hawaii (Halstead, 1992). A new manifestation of severe dengue illness resulted, dengue haemorrhagic fever, first reported in the Philippines then later in Thailand during the 1950s (Halstead, 1992).

The discovery of the role of *Aedes aegypti* in the transmission and spread of yellow fever and the subsequent isolation of the virus and creation of an effective yellow fever vaccine introduced the concept of mosquito control as an effective measure to disrupt yellow fever transmission. Subsequently the International Health Board and the Rockefeller Foundation instituted mosquito control strategies including the use of a larvicidal, Paris Green, throughout the USA and Central and South America (Stapleton, 2004). These techniques were soon applied to malaria control and during the years from 1924 to 1925, funding

for malaria prevention through the strategy of mosquito control doubled (Stapleton, 2004). The success of this programme in Italy during the 1920s set the stage for the global use of mosquito control in the prevention of malaria. The Second World War prompted the creation of the Rockefeller Foundation Health Commission in 1942 to support national defence and in particular malaria control for U.S. forces. The need for lousicides to combat typhus ushered in a new insecticide developed by the Swiss firm, Geigy, called dichlorodiphenyl-trichloroethane (DDT) (Stapleton, 2004). Led by Fred Soper, the Rockefeller team demonstrated the effectiveness of DDT as a lousicide and in disrupting typhus epidemics. DDT was soon used in aerial and ground spraying for Allied Forces during a malaria outbreak in Italy and was found to be a highly effective larvicide with a long environmental persistence. DDT subsequently became a key component of the World Health Organization's global malaria eradication campaign in 1955 (Stapleton, 2004). This campaign resulted in the elimination of both the malaria mosquito vector and *Aedes aegypti* throughout South America and the virtual elimination of malaria, yellow fever and dengue throughout the Americas. A reassessment of this global strategy by the WHO and the growing concerns of the environmental effects of DDT led to the end of the use of DDT as a mosquito control larvicide in 1969 (Nájera, 2001). The cessation of DDT-based mosquito control programmes in the Americas and the social disruption that resulted from the Second World War allowed the spread of DENV in Asia, the reintroduction and resurgence of *Aedes aegypti* throughout the Americas, and, consequently, resurgence of DENV, particularly South-East Asian strains, in the Americas.

The first two dengue pandemics were characterized by epidemics that produced severe outbreaks of fever, headache and myalgias, a clinical syndrome termed dengue fever. As waves of DENV-1 to -4 spread throughout the human population, especially in Asia, DENV adapted to be able to reach virus levels during a course of infection that allowed mosquitoes to become infected, thereby ensuring continued transmission of the virus. Chapter 8 discusses variation among vector species in their susceptibility to dengue and the potential selective effects of such

variation on viral replication; however, high levels of co-circulation among serotypes also posed a challenge for the persistence of each serotype. Consider a DENV-2 strain entering a population that had a high degree of pre-existing antibody to an established DENV, such as DENV-1. Pre-existing DENV-1 antibody, though not neutralizing, would under ordinary circumstances have provided significant heterotypic neutralization of DENV-2, potentially reducing viral levels in infected humans and thereby interrupting mosquito transmission. Thus, the presence of high levels of infection by multiple serotypes imposed significant selection for viruses that, via mutations in the E protein coat and changes in specific epitopes, were able to either fully escape the effects of heterotypic neutralization, or as is currently thought to be the case, to utilize these subneutralizing antibodies to enhance infection. This phenomenon of viral replicative enhancement due to subneutralizing heterotypic antibody is known as antibody-dependant enhancement (ADE) (Halstead, 1992). Since ADE results in higher viral loads, viruses with a particularly high tendency towards enhancement should have a selective advantage (Cummings *et al.*, 2005; Ferguson *et al.*, 1999a,b).

The ability of all DENV serotypes to utilize pre-existing heterotypic flavivirus antibody to enhance infection is a unique feature of DENV that is particularly common among South-East Asian strains. The tendency to be enhanced by heteroserotypic antibody distinguishes DENV from all other flaviviruses, and is the primary basis of DENV pathogenesis in severe dengue illness. During the third pandemic, this tendency of DENV to be enhanced in secondary dengue infection resulted in the clinical manifestation of a previously unrecognized sequelae of DENV infection – severe haemorrhagic disease and plasma leakage (Halstead *et al.*, 1963). First described as Philippine and Bangkok haemorrhagic fever during the 1950s, it is now recognized as dengue haemorrhagic fever (DHF).

Studies of dengue in Thailand

During the 1950s, the South-East Asian Treaty Organization (SEATO), in response to a cholera outbreak occurring throughout Asia, created a number of laboratories comprised of host-country

and US scientists in Thailand, Malaysia, Bangladesh and Pakistan. The Thailand laboratory named the SEATO General Medical Research Project located in Bangkok, later re-named the Armed Forces Research Institute of Medical Sciences (AFRIMS) in 1977, formed a still ongoing 50-year relationship of Thai-US collaborators in the study of tropical infectious diseases. The discovery that Bangkok was experiencing an outbreak of a new clinical manifestation of dengue infection, dengue haemorrhagic fever by both Thai and US scientists, allowed the ongoing study of dengue in Thailand that spanned over a half of a century producing many of the seminal concepts of dengue virus transmission and disease severity. Early studies on DHF in Thailand established this as a unique clinical syndrome of DENV infection. Careful clinical studies of hospitalized children in Bangkok, Thailand demonstrated the clinical severity of DHF in producing thrombocytopenia, leucopenia, coagulopathy and plasma leakage (Nimmannitya *et al.*, 1969). Studies on DHF pathogenesis in the 1960s revealed its unique features in being largely a phenomenon of secondary DENV infections or in primary infection of infants, a function of declining maternal DENV antibody (Halstead, 1988). Classic studies in Thai children first established the role of enhancing antibody in the peripheral blood mononuclear cells of children in producing severe dengue illness and DHF (Kliks *et al.*, 1988). Prospective studies in hospitalized Thai children and in long-term cohort studies demonstrated the importance of dengue viral load and the T-cell response in determining dengue severity, the diversity of all four dengue serotypes circulating spatially and temporally in a well-defined geographic area and the role of subclinical dengue infection and its contribution to the overall burden of dengue illness (Anderson *et al.*, 2007; Endy *et al.*, 2002a,b, 2004).

The next step...

Despite our increased understanding of both virological and host factors of DENV and human infection, many questions remain about the virus-host interactions that result in severe dengue illness. At present, viral virulence factors and the mechanisms by which the South-East Asian strains produce DHF are not well characterized.

The role of the non-structural proteins in viral replication and escape from the host's innate immunity are discussed in detail in Chapters 3 and 7, respectively. Host genetic factors that predispose to DHF (discussed in Chapter 5), the role of heterotypic antibody in protection and enhancement of infection (discussed in Chapter 14) and a functional assay to detect this antibody are important scientific questions that are being explored. Many challenges remain in the quest to control DENV, including prediction of dengue transmission dynamics (Chapter 10), development of tractable, informative animal models (discussed in Chapter 6), development of novel therapies (Chapters 13 and 14), and the ultimate challenge (Chapter 12), development of a DENV vaccine that provides durable long-lasting protective immunity against infection.

Dengue has emerged in the last 60 years as a global health problem producing severe morbidity and mortality across the subtropical and tropical regions of the world. Considering that much of the emergence of dengue epidemics is due to population growth, continued spread of the vector *Aedes aegypti* and urbanization of the developing world, dengue will continue to grow as this century's most important public health problem.

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