Monthly Focus: Anti-infectives

Patents related to dengue virus infection

Mohamad Warda, Rory M Marks & Robert J Linhardt
1Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA
2Division of Medicinal Chemistry, Department of Chemistry and Department of Chemical and Biochemical Engineering, University of Iowa, Iowa City, IA 52242, USA

This review discusses patents relevant to dengue virus infection. Dengue virus is a mosquito-transmitted flavivirus that usually causes an acute self-limited febrile illness, but may result in more severe life threatening disease. The lack of animal models that replicate features of the human disease has inhibited developing an understanding of the pathophysiology of dengue virus infection and consequently, limited investigation into potential therapeutics. However, there has been considerable progress in vaccine development and recent insights into the identity of the cells infected during the course of dengue virus infection and characterisation of cell-surface receptors used to mediate infectivity, have provided important information with therapeutic implications. There is no treatment for dengue virus infection and no vaccine is yet available, new approaches are urgently needed.

Keywords: antivirals, arbovirus, dengue, flaviviridae, flavivirus, haemorrhagic fever virus, monoclonal antibodies, polyamines, protease inhibitors, RNA polymerase inhibitors, vaccines


1. Introduction

1.1 Phylogeny

Dengue virus is a member of the genus flavivirus within the family Flaviviridae [1]. The genus and family are named for the prototype yellow fever virus (latin flavus translates as yellow). Yellow fever and dengue virus infection represent the first and second human diseases identified as being caused by filterable agents (i.e., viruses) [2]. The flaviviridae are divided into three genera: pestiviruses (including the important agricultural animal pathogens bovine viral diarrhoea virus, classical swine fever virus and border disease virus), hepacivirus (hepatitis C virus) and flaviviruses. Over 70 flaviviruses have been identified, approximately half of which can cause human disease [3]. The dengue virus group is divided into four closely related species (dengue 1 – 4), based on serological characteristics and genomic sequence. Most flaviviruses, including dengue virus, are also classified as arboviruses, being transmitted by the bites of infected arthropods. Readers are referred to [4] for a comprehensive review of the Flaviviridae family. Dengue virus is further classified as a haemorrhagic fever virus [4], a phylogenetically disparate grouping of viruses that cause febrile illnesses associated with severe vascular damage.

The lack of specific knowledge about the pathophysiology of dengue virus infection leads to consideration of whether existing information about the pathophysiology of related viruses could be used to infer potential therapeutic targets for dengue virus. For example, the nucleoside analogue ribavirin (1-β-D-ribo-furanosyl-1,2,4-triazole-3-carboxamide) is used to treat hepatitis C [6] and does inhibit dengue virus replication in vitro [7]. However, dengue virus and hepatitis C virus have only distantly related genomes [5], ribavirin is not considered to have useful anti-dengue activity in vivo [8], and the utility of this approach is very speculative. Viral proteases are appealing targets for the development of antiviral drugs and the
Patents related to dengue virus infection

hepatitis C and dengue virus NS3 proteases do have some common structural characteristics; however molecular models also predict distinct differences in interactions with substrates and cofactors that will likely limit generating crossreactive inhibitors [9,10]. Recent studies have revealed that the major ectodomain proteins of alphaviruses and flaviviruses have similar structures, despite a lack of sequence homology [11]. These proteins are responsible for the critical initial events in viral infectivity, target cell binding and membrane fusion and their similarities may lead to broader evaluation of common inhibitors.

1.2 Mosquito vector
Dengue virus is transmitted to humans by the bite of *Aedes* genus mosquitoes [12]. The species, *Aedes aegypti*, is by far the most important disease vector for dengue virus [13]. Female mosquitoes acquire infection by biting and ingesting blood from a viremic human subject. Virus infects mosquito mid-gut epithelial cells, is released into the haemocyte and then causes infection of salivary glands with persistent secretion of virus into saliva. Dengue virus does not cause significant damage to the vector species and infected mosquitoes transmit virus to humans in saliva that is injected into the skin during the process of probing for a bloodmeal [14]. Larvicidal spraying was previously very successful at controlling dengue virus transmission, although attempts at reintroduction have failed (see below). New larvicides or compounds targeted at the mosquito that block critical aspects of virus–mosquito interactions, or that have a specific antiviral effect, have potential but are beyond the scope of this review.

1.3 Epidemiology of infection
Transmission of dengue virus infection occurs in regions that are warm enough for propagation of the mosquito host (30°N – 20°S latitudes) and have human habitats in proximity to the collections of standing freshwater required for mosquito breeding. These conditions exist throughout the tropical and subtropical developing world [15,16]. Two and a half billion people in over 100 countries are at risk of infection, with tens of millions of infections per year, mostly in children [17]. The modern history of the epidemiology of dengue virus infection is linked to the initial success and then failure of *Aedes* mosquito control programmes [16,19,20]. After World War II, widespread larvicidal spraying achieved remarkable reductions in the incidence of dengue virus infection. Subsequent dismantling of these programs led to failure to control mosquito populations and recent alarming increases in the incidence of infection [16,20]. Dengue virus infection is generally considered to be pandemic in scope in the tropical developing world. Dengue virus also has a considerable and underappreciated economic cost; a study of age-related loss of productive life due to disease estimated that in Latin America the economic impact of dengue virus infection is of the same order of magnitude as other common diseases, such as tuberculosis, malaria and hepatitis [21,22].

Programmes aimed at regaining control over dengue virus infection by means of public health education and reintroducing larvicidal spraying have been unsuccessful; a programme instituted in Puerto Rico with widespread support has not had any impact on alarming increases in the incidence and frequency of regional dengue virus epidemics [23]. Attempts at maintaining previous levels of control over *Aedes* mosquito populations and dengue virus have failed for reasons that are not completely clear, but probably involve population growth with increasing urbanisation and associated substandard housing and inadequate piped water, the high density of larval-infested water containers and other non-biodegradable materials that trap water, and increased mobility facilitating dispersal of infected humans and mosquitoes [24]. None of these factors will be readily overcome and represents a staggering and overwhelming public health challenge to the developing world [23,25]. The failure of public health mosquito-control based programmes has led to increased imperius to develop an effective vaccine to prevent infection and effective drugs to treat severe infection.

1.4 Clinical disease
Uncomplicated dengue virus infection causes an acute febrile illness [26]. Within 3 – 15 days of a mosquito bite, illness manifests with fever lasting 2 – 6 days, terminating with a crisis and defervescence. Specific clinical features accompanying the fever are variable but often include severe debility, headache, retro-orbital pain and photophobia, vertigo, dysgeusis, myalgia, bone pain, generalised lymphadenopathy, anorexia and nausea and a rash. An evanescent macular erythematous rash is often observed at the onset of disease and a secondary maculopapular puritic erythematous rash may occur later in the course of the disease. These persist for 1 – 5 days and may be accompanied by a second febrile period. Leukopenia (both neutropenia and lymphopenia) and thrombocytopenia are commonly observed but do not reach clinically significant levels. Remarkable clinical research from the US Army Medical Corps in the Philippines in 1926 and 1931 defined the clinical characteristics of dengue virus infection summarised here, as well as many important features of mosquito infection and transmission [13,27].

After World War II an epidemic clinical syndrome was recognised, characterised by fever, haemorrhagic manifestations and shock [28-30]. This was later shown to be caused by dengue virus, leading to the understanding that dengue virus caused disease over a wider clinical spectrum of severity than previously realised, with severe complex disease being characterised by progressively more severe vascular pathology. A World Health Organization classification of dengue infection has been developed, with uncomplicated dengue fever; dengue haemorrhagic fever (DHF), characterised by capillary fragility, plasma leakage, thrombocytopenia and potential haemorrhage;
and dengue haemorrhagic shock syndrome (DHSS) additionally characterised by cardiovascular collapse [31,32]. Autopsy studies of DHF patients reveal widespread petechial haemorrhage, indicating that generalised microvascular damage is a common and important pathological feature [33]. Involvement of primary and secondary immune organs is also a common feature in DHF [34,35].

Complicated dengue infection usually occurs when multiple serotypes of dengue virus are circulating within the same geographic region and when infected patients have pre-existing (but non-neutralising) antibodies to dengue virus. These antibodies develop as a result of prior infection with a different serotype [36-44]. Non-neutralising antibodies enhance, rather than inhibit, the in vitro infectivity of dengue virus and other microorganisms, a process known as antibody-dependent enhancement (ADE) of infectivity [45,46]. Enhancing antibodies act as a bridge that binds dengue virus to cells expressing Fc receptors: the antigen binding region of antibody binds to (but does not inactivate) the virus, while the Fc region of antibody binds to Fc receptors on target cells. ADE has been invoked as the mechanism that explains the association between clinically severe infection and pre-existing non-neutralising antibodies in patients with DHF. Not all cases of DHF are associated with detectable antidengue antibodies [47,48], yet ADE by non-neutralising antibodies is the only currently credible explanation for the occurrence of the great majority of cases of DHF. Concerns about this phenomenon have limited vaccine development, as a safe vaccine will have to provide effective protection against all four serotypes of dengue virus.

1.5 Pathophysiology

The lack of animal models has been a great impediment to developing an understanding of the pathogenesis of dengue virus infection. Humans, lower primates and mosquitoes are the only known natural hosts of the infection [26]. Rodents can be infected under artificial conditions but do not replicate features of the pathophysiology of human infection [26,49].

Subhuman primates are readily infected by dengue virus and the initial viral dissemination, as well as the development of viremia, does replicate findings in humans [50-52]. However, most infected subhuman primates do not manifest clinical disease during viremia, limiting their utility as models. At this time, data from studies of infected humans, supplemented by studies in monkeys, human ex vivo organ cultures and in vitro tissue culture models, have provided important but limited insights into pathogenesis.

Dengue virus antigens can be identified within specific skin cells in rhesus monkeys at the site of a bite from an infected mosquito [26]. The movement of these dengue-positive cells into the dermis suggested these initially infected cells were Langerhans-type dendritic cells, confirmed in a later study using human skin explants [53]. This is concordant with the known sentinel role of dendritic cells in the immune system; to ingest and process antigens (including many viruses) and to present antigens to lymphocytes for induction of a protective immune response [54,55]. Infection of dendritic cells also explains the initial spread of dengue virus from the skin bite-site to regional lymph nodes and possibly beyond. Activated dendritic cells migrate from their peripheral location, through the afferent lymphatics, to regional lymph nodes, where they accumulate in T lymphocyte rich paracortical regions and prime T and B lymphocyte antigen-responsiveness [56] and possibly other cells.

Following dengue virus accumulation in lymph nodes, patients become viraemic, with the virus detectable free in the blood, as well as associated with blood mononuclear cells [57]. One study indicated that B lymphocytes are the primary circulating infected cell type [58]. Studies of infected tissues have consistently indicated that macrophage-like cells are most commonly associated with infection [15]. However, tissue-based studies have been extremely limited compared with analysis of blood and mostly restricted to the small proportion of patients dying from DHF. Dengue virus and viral RNA has been sporadically isolated from a wide variety of tissues in these patients and there is little indication that viral dissemination is restricted in terms of organs infected [24,44,59]. Skin rash is a prominent clinical feature and in a single reported case, a patient administered with a live attenuated dengue virus vaccine candidate and who developed viremia and a rash, also showed detectable levels of dengue virus antigen within lesional tissue dendritic cells [53]. Extrapolating from these findings and taking into account the diverse and variable symptoms and clinical signs reported in dengue fever, the available data suggest infection is widely disseminated throughout the body, with variations in clinical presentation attributable to individual heterogeneity in host responsiveness. The principal clinical feature of uncomplicated dengue virus infection is fever. The febrile response is due to systemic release of cytokines that possess endogenous pyrogenic activity [60]. Increased circulating levels of these cytokines have been associated with dengue virus infection [61] and these probably originate from infected macrophages and other infected cell types.

Explaining the differences in clinical presentation between the majority of patients with uncomplicated dengue fever and the small proportion of patients with DHF is a fundamental but unanswered question. A recent study demonstrated that patients with DHF had significantly higher titres of circulating virus than did patients with uncomplicated disease [48]. It is possible that disease severity relates simply to ADE-mediated increased viral load. However, ADE could fundamentally alter the virus-host interaction, with the increased viral titre in DHF being an epiphenomenon. Genetic variation in dengue viruses, resulting in increased virulence could also relate to the development of DHF [62] and there may also be genetic and acquired variations in host susceptibility.

The presence of vascular involvement, indicated by leakage of plasma and haemorrhage, is the major clinical characteristic differentiating DHF from uncomplicated disease [33]
and implicates the vasculature as having a central role in mediating the pathophysiology of DHF. Pathological studies of skin lesions have revealed microvascular endothelial pathophysiology (swelling), interstitial oedema and a perivascular infiltrate of inflammatory cells [63]. Some cytokines cause retraction of vascular endothelial cells leading to plasma leakage [64-67] and release of vasoactive cytokines from infected target cells, such as macrophages, has been suggested as the cause of vascular disease in DHF [68]. However, cytokines do not cause the extensive vascular disruption required to cause haemorrhage and although it has been difficult to demonstrate infection of vascular cells in patients with DHF, it seems most likely that direct infection of vascular endothelial cells (as occurs with many other microorganisms) is the cause of vascular disease in DHF. Vascular disruption could be caused either by a direct cytolytic effect of dengue virus or alternatively infected vascular cells could be destroyed by elements of the host inflammatory and immune systems. Dengue virus infection is associated with apoptotic death of vascular endothelial cells [69] in vitro and other cell types in vivo [70,71] and in vivo [72] and it is possible that virus-induced apoptosis could contribute to the pathology of simple and complex infection and may represent an important therapeutic target.

The skin, lymph nodes and other organs of the immune system (including the liver) and vasculature, are likely targets for dengue infection; however, there is little understanding of the specific nature of the interaction of dengue virus with any target cells and tissues. The infectivity of viruses is initially dependent on their ability to bind to the surface of a target cell [73]. Binding is followed by membrane fusion and leads to productive infection in suitable host cells [1]. The particular cell and tissue tropism exhibited by a virus is often defined and restricted by the specificity of the receptor-like interaction that occurs between a viral ectodomain molecule and a receptor molecule, expressed by a specific target cell type. ADE can not explain cell binding and infection of cells in patients without dengue antibody, nor can it explain the ability of dengue virus to infect cell types that do not express Fc receptors [74]. The glycosaminoglycan heparan sulfate has been identified as a potential dengue virus receptor in in vitro studies [75]. The known role of glycosaminoglycans as receptors for many other microorganisms and the extensive distribution of glycosaminoglycans on cells throughout the body (matching the extensive dissemination of dengue virus in infected patients) suggest heparan sulfate is a physiologically relevant receptor molecule [76]. Unidentified proteins have also been characterised as potential dengue virus receptors [77], however interactions, if any, between glycosaminoglycan and protein receptor molecules (as occurs with other viruses), has not been clarified for dengue virus [78,79]. Binding of the virus to receptors represents the primary extracellular interaction of virus and target cell and may be an optimal opportunity for a potential antiviral to disrupt infectivity. Similar considerations have led to development

and evaluation of anti-HIV compounds that disrupt HIV gp120 envelope protein (fusion) interactions [80] and an antirhinovirus therapeutic [81]; the dengue virus envelope protein binding and fusion reactions are a similarly appealing target for drug development.

1.6 Immune response

The close correlation between clearing of viremia, resolution of symptoms and appearance of IgM antidengue antibodies in the circulation has focused much attention on the adaptive humoral immune response to dengue virus [24,82]. The IgM response is quickly followed by the appearance of IgG antibodies. IgM antibodies wane over a 2-3 month period but the IgG response persists. The presence of an acquired specific IgG response to infection is associated with long-lived immunity to the infecting serotype but, as discussed above, does not protect against infection with other serotypes of dengue virus. T lymphocyte responses to dengue virus antigens can also be detected in the convalescent phase after infection and multiple epitopes expressed by the envelope protein, as well as by other structural and non-structural proteins, stimulate T lymphocytes [83]. Activated T lymphocytes directly release pro-inflammatory cytokines, as well as stimulating other cells, such as macrophages, to release cytokines. Some of these cytokines can cause increased vascular permeability, a characteristic feature of DHF leading to the suggestion that in secondary dengue virus infection it is the activation of memory T lymphocytes that accounts for the pathophysiology of DHF [68,84].

The innate immune system comprises the protective responses of a host to infection that do not feature the somatic mutation, maturation of antigen-specific immune responsiveness and immunological memory, that characterises adaptive (antibody and T lymphocyte-mediated) immunity [85,86]. These include natural killer lymphocytes, phagocytic cells, such as monocytes, that can kill microorganisms, soluble mediators such as pro-inflammatory cytokines and complement that can be directly cytolytic, activate inflammatory cells and opsonise microorganisms for ingestion and killing by phagocytic cells and cell-associated antiviral mediators such as interferons. Innate immune responses immediately provide effective, broad, but relatively non-specific protection and presumably limits the extent and severity of infection until neutralising IgM antibodies appear. A recent study demonstrating that transgenic mice deficient in interferons (IFNs) and IFN receptors were susceptible to dengue virus infection reinforces the important role of IFNs in controlling the extent of dengue virus infection [87,88]. There have also been many studies of the roles of different types of leukocytes in killing dengue virus and studies demonstrating activation of the complement system in patients with dengue virus infection [24]. There has been a recent general resurgence of interest in the innate immune response, with novel mechanisms being defined and a realisation that effective activation of adaptive immunity requires prior activation of innate immune mechanisms [85,86]. However, in the

1130 Expert Opin. Ther. Patents (2002) 12(8)
absence of adequate animal models it has not been possible to identify which of these mechanisms is important in the control of dengue virus infection.

1.7 Vaccine development

Inactivated microorganisms are the basis of many vaccines and a potential monovalent vaccine candidate has been generated from inactivated dengue virus and remains under active investigation [90], as do dengue structural proteins expressed as recombinant subunits [91]. DNA-based vaccines, that induce an immune response by introducing a cDNA-cassette into recipients, which then leads to expression of selected viral proteins, are under development but have not yet been evaluated in human trials [92].

In general, it has been difficult to generate a long-lasting immune response from a practical immunisation schedule using inactivated dengue virus or dengue virus proteins and most vaccine research has focused on developing tetravalent live (i.e., active against all four dengue virus species and replication competent) virus vaccines. Live virus vaccine candidates have been developed by traditional laboratory attenuation, serially passaging viruses through animals or cultured cells [93]. A more recent approach has been to take advantage of knowledge of specific nucleotide sequences that correlate with a virulent phenotype and to introduce specific virulence-attenuating mutations into the dengue virus genome [95]. A particular advantage of the molecular approach to attenuation is that it provides the opportunity to insert multiple mutations or to delete sequences [18], as a guard against reversion of the mutant to a virulent phenotype.

Chimeric viruses have also been developed, combining limited structural regions of a dengue viral genome required to generate a desired immune response into the genome of virulence-attenuated dengue viruses [94], related flaviviruses [95] or other viruses such as poxviruses [96]. A yellow fever–dengue chimaera seems a particularly promising approach as it makes use of a clinically well-tested and reliable vaccine strain of yellow fever virus [95].

The necessity to simultaneously induce long-lasting immunity to all four dengue virus serotypes to prevent enhancement of infectivity by non-neutralising antibodies (discussed above), has been an impediment to vaccine development. The principle of DNA shuffling could result in developing a single DNA vaccine cassette that leads to protection against multiple dengue serotypes [148]; however, this approach is unproven. Most approaches to vaccine development have had to incorporate mixtures of four different immunogens, one for each dengue virus serotype, considerably complicating development and evaluation. Evaluation of tetravalent live vaccines requires particular attention to quality control, prevention of reversion to a virulent phenotype and development of excessive clinical reactogenicity, as well as development of an effective immune response. In general, it seems that a vaccine is still years away from clinical deployment [96].

1.8 Virus structure and function

The flavivirus genus encompasses viruses that exhibit the same overall genomic organisation, a high degree of sequence homology and shared antigenic epitopes [1,97]. Due to these similarities, structural studies of individual flaviviruses have been widely applied to other members of the genus and this approach has yielded valid and useful information. A recent study used cryo-electron microscopy to reveal the structure of dengue virus [98] and yielded data compatible with the X-ray crystallographic structure of the envelope protein of the flavivirus tick-borne encephalitis virus [99]. The mature dengue virus consists of a single positive strand of RNA surrounded by the virus-encoded nucleocapsid protein and is ~30 nm diameter. The nucleocapsid is surrounded by a lipid envelope derived from the host cell and probably acquired by the nucleocapsid budding through the membrane of the endoplasmic reticulum. The virus-encoded envelope and membrane proteins are embedded in this lipid envelope and the whole virion is ~50 nm in diameter [1,98,100].

The genes encoding the flavivirus structural proteins are located at the 5' end of the genome and are followed in sequence by the non-structural proteins. A single intact polyprotein is generated, with individual proteins then derived from the polyprotein by proteolytic processing [1]. The 13 kDa capsid protein is expressed at the amino-terminus, of the polyprotein and contains a large proportion of basic amino acids that are probably important in mediating ionic interactions between capsid protein and the RNA genome that it encapsulates [101].

The membrane protein is generated as a 22 kDa pre-membrane precursor that undergoes cleavage during virus development to an 8 kDa mature form [101]. The mature membrane protein contains a hydrophobic region at its carboxy-terminus which likely accounts for tethering to the viral lipid membrane, exposing only a short amino-terminal region. Cleavage of pre-membrane to membrane protein is crucial to the generation of mature infectious virus and closely correlates with release of the virus from the host cell. It is believed that the function of the pre-membrane protein is to associate with the envelope protein and prevent premature activation of acid-triggered envelope protein fusion during development and passage of virions through the cell [102]. Cleavage of the pre-membrane protein releases the envelope protein from this inhibitory control, leaving mature virus capable of undergoing the fusion reaction that is a critical component of its ability to infect target cells. Recent cryo-electron microscope studies of dengue virus [98] and recombinant subviral particles of tick-borne encephalitis virus [103] have confirmed that the mature membrane protein maintains a close association with the envelope protein and may retain a role in regulating the conformation of the envelope protein before and during fusion.

The envelope protein is thought to express the structural motifs essential for the initial interactions that are critical for mediating infectivity, target cell binding and membrane...
Patents related to dengue virus infection

fusion and considerable experimental data now support this hypothesis [1,24,26]. A major increase in our understanding of the structure and function of flavivirus envelope proteins was achieved with the publication of a high-resolution X-ray crystallographic structure of most of the ectodomain of the envelope protein of the flavivirus tick-borne encephalitis virus [99]. This was followed by a cryo-electron microscope study of dengue virus, which provided detailed molecular structural organisation of the entire virion [98]. These and other studies [11] reveal that flavivirus and alphavirus envelope proteins occur as a head-to-tail dimer lying flat in the plane of the viral surface. This structural organisation is in distinct contrast to the spike-like fusion proteins projecting away from the surface of other viruses, such as influenza virus and HIV.

Flavivirus envelope proteins are folded into three distinct domains [99]. Domain I forms a central β-barrel and is generated from both amino- and carboxy-terminal sequences. Domain II forms an elongated finger-like structure that incorporates the motif responsible for membrane fusion at its distal tip [104]. Membrane fusion of flaviviruses is triggered by exposure to the acid environment of endosomes that are entered following receptor-mediated endocytosis. However, dengue virus has also been shown to be directly taken up by cells at the cytoplasmic membrane [105]. Fusion results in merging of the viral envelope into target cell membranes, leading to introduction of the virus into the cytosol of the target cell and is a prerequisite for releasing the viral genome and initiation of viral replication. Flavivirus fusion involves trimerisation of the envelope protein [106]. Exposure to acid pH leads to conformational changes that weaken dimer interactions, exposing the metastable fusion motif, while strengthening lateral interactions that generate the homotrimeric envelope protein form.

Domain III of the envelope protein is generated from carboxy-terminal sequence and is in the form of an immunoglobulin-like (IgC) module that is commonly associated with structures that have an adhesive function. Many other features also consistently indicate that domain III accounts for binding to target cells; it projects away from the surface of the virion and is externally accessible, it incorporates an Arg-Gly-Asp sequence in some flaviviruses (a common adhesive motif), it is the binding site for many of the antibodies that prevent infection and it incorporates the glycosaminoglycan-binding motifs that probably account for binding to heparan sulfate expressed on the surface of target cells [75,99].

1.9 Genomic organisation and non-structural proteins

Dengue virus, like other flaviviruses, has a genome consisting of a single positive-sense strand of RNA 11 kb in length. The RNA commences at its 5' end with a methylated nucleotide cap structure [107,108]. Unusually for a RNA virus, the 3' terminus is not polyadenylated but this region is, however, required for viral replication [93]. Flanked by short 5' and 3' non-coding regions, the dengue genome is translated as a single open reading frame, generating a polyprotein that is subsequently cleaved into individual structural (incorporated into virions) and non-structural proteins. The capsid, membrane and envelope proteins are translated in that order and have been discussed above. The capsid protein is translated with a carboxy-terminal hydrophobic segment, probably responsible for initial membrane association that is later removed. The membrane and envelope proteins have carboxy-terminal hydrophobic regions that are retained in the mature proteins and are responsible for tethering into the virus lipid membrane. Following translation of capsid membrane and envelope proteins, seven non-structural proteins are generated: NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5. NS3 is a trypsin-like serine protease responsible for cleavage of the polyprotein at the junctions between NS2A-NS2B, NS2B-NS3, NS3-NS4A, NS4B-NS5 and possibly also for cleavage of the hydrophobic carboxy-terminus of the capsid protein [9,10,97,109,110]. NS3 also has helicase activity, which hydrolyses nucleotide triphosphates while unwinding double-stranded duplexes into single strands [109]. Other junctions are cleaved by the host cell signal peptidase [97]: capsid-pre-membrane, pre-membrane-envelope protein, envelope protein-NS1 and NS4A-NS4B. The mechanism for cleavage of NS1-NS2A and cleavage of pre-membrane to membrane proteins are not known.

All the non-structural proteins appear to be required for successful replication of the virus and in general, dengue virus has defied attempts at selective deletion of genetic material or insertion of genetic tags. Furthermore, a correlation between genomic sequence and virulence has indicated virulence determinants scattered through the genome, including the region encoding non-structural proteins, signifying their potential importance [62]. These features indicate that the non-structural proteins, could be appealing targets for development of highly virus-specific drugs. However, compared with the structural proteins incorporated into virions, the functions of the non-structural proteins are not well understood. NS1 is one of special interest, notwithstanding its unknown function, as it is found exposed on the external surface of some dengue virus infected cells and generates a protective immune response. NS1 is found in the form of a dimer [24,111,112]. NS2A has no known function [97]. NS2B is found as a complex with the protease NS3 and is necessary for proteolytic activity to occur but is not itself a protease [97,113]. The functions of NS4A and NS4B are unknown. NS5 is a RNA polymerase, as well as expressing methyltransferase activity that may account for methylation of the 5' RNA cap structure [114,115]. Most information exists for the NS3 protease and helicase.

1.10 Replication

Infection of a target cell and fusion with endosomal membranes is followed by the uncoating and release of viral RNA into the cytosol. This single positive strand of RNA serves both to initiate translation of the polyprotein on ribosomes and as the template for generation of a replicative
complementary negative sense strand [1,97]. The negative sense RNA is used as the template for the synthesis of multiple positive sense strands that are then incorporated into new virions. The specific roles for the helicase activity of NS3 and the RNA polymerase activity of NS5 in viral replication have not been defined, however, both are found in association with replicative forms. The virus develops by the positive sense RNA genome becoming coated by the capsid protein and then budding into the rough endoplasmic reticulum (ER). Budding leads to the nucleocapsid becoming coated by the ER membrane, which already contains the viral pre-membrane and envelope proteins. Although flavivirus budding has been observed to occur at the cytoplasmic membrane, most studies indicate that this occurs predominantly at the ER. The virus is released from the cell surface by exocytosis.

2. Discussion

2.1 Vaccines

A formalin-inactivated Japanese encephalitis (JE) virus vaccine is effective and in clinical use, prompting similar development of an inactivated dengue 2 virus vaccine candidate [201]. This consists of dengue virus grown in Vero cells, highly purified, then inactivated with formalin. This preparation has protective activity in mice and primates [90] but no human data are published.

Patent [202] describes tetravalent recombinant dengue virus envelope proteins that generate a virus-neutralising immune response but no published data are available.

Patents [203-207] describe the generation of laboratory-attenuated dengue viruses of all four serotypes and evaluation of clinical reactogenicity and immune responsiveness in humans. Administration of tetravalent viruses generated an immune response to all four serotypes in 40% of subjects.

The availability of infectious plasmid cDNA clones of the entire dengue virus genome provides the opportunity to engineer specific virulence-attenuating mutations, including multiple mutations as a guard against reversion to wild-type virulence. Patent [208] describes the generation of a mutant dengue 2 virus with a 3'-non-coding region stem-loop structure substitution that is replication defective and is being evaluated as a vaccine candidate.

Chimeric viruses offer the opportunity to engineer regions of the dengue virus genome encoding structural proteins for induction of an immune response, combined into other viruses with virulence-attenuating mutations and other desired characteristics. Patent [211] describes the generation of a modified vaccinia virus designed to express dengue virus proteins. Patent [212] describes the generation and evaluation of a chimeric virus generated from tick-borne encephalitis virus and dengue-4 virus. Patent [209] is of particular interest as it describes a chimera generated from a clinically well tested and reliable vaccine strain of yellow fever virus and structural regions of dengue virus and other flaviviruses of interest. Many other chimeras are in development and are reviewed in [96].

2.2 Antiviral drugs

Once dengue virus enters the body through a mosquito bite, binding to cellular receptors represents the first interaction that can be targeted and may represent an optimal opportunity to disrupt infectivity. Subsequent targets include viral fusion and entry, uncoating, replication and release. The best molecular targets are elements unique to the virus or critical to supporting its life cycle. Such considerations have led to development and evaluation of a number of antiviral therapeutics, including anti-HIV compounds that disrupt HIV gp120 envelope protein (fusion) interactions and the HIV protease [144,145].

A useful therapeutic for treating dengue infection must:

- be effective
- be devoid of undesirable activities
- have a high therapeutic index
- have low toxicity
- be bioavailable
- be inexpensive

Currently, the best medicinal chemistry approach for designing an effective anti-infective is to begin with the structures of the already known agents that block envelope protein binding to target cells and that inhibit molecular processes critical for infection and replication. The non-vaccine based approaches described below are listed in order of their perceived therapeutic potential; none have demonstrated in vivo clinical activity or are in human clinical trial and are presented more as conceptual targets or as illustrating potential avenues for future research, not as specific examples of especially promising drug candidates.

2.2.1 Serine protease inhibitors

Dengue virus has a critical protease coded by the NS3 gene, related to the hepatitis C virus protease [1,11]. The successful treatment of HIV infection with protease inhibitors [117] suggests this is a potential approach for developing dengue antivirals.
Protease inhibitors, such as 6-hydroxy-2-isopropyl-1,6-dihydro-2H-pyridin-3-one (1) have been proposed as antiviral agents. Patent [210] describes the potential use of compound 1 as a viral serine protease inhibitor.

2.2.2 RNA-dependent RNA polymerase
Enzyme activities unique to the virus, such as RNA dependent RNA polymerase, represent an excellent potential target for antiviral therapy. Patent [213] describes a method for treatment and prophylaxis of infection caused by *Flaviviridae* based on administration of rhodamine derivatives and analogues, including tri- and tetracyclic rhodan alkanoic acid and benzoic acid. Compound 2 shows *in vitro* inhibition of RdRNP but no antiviral activity or *in vivo* activity has been reported.

In compound 2, R is H or alkyl; and n is an integer from 0 – 4; R is H or -R₃COOH (where R₃ is an unsubstituted, branched or straight chain, saturated or unsaturated phenyl, substituted or unsubstituted phenylalkyl, aliphatic group branched or straight chain, saturated or unsaturated having 1 – 6 carbon atoms, an unsubstituted or substituted heterocycle (such as oxazole, oxadiazole, pyridine, pyrimidine, pyrazole, triazole, pyridazine, 1,3-oxathialane, thiazole, thiazole, imidazole, tetrazole, pyrrole and triazine)). X is S, O or N(R₄) (where R₄ is H or alkyl or 1 – 5 carbon atoms). R₂ is an unsubstituted or substituted phenylalkyl, phenylalkenyl, phenylnaphthalenyl, biphenylalkyl poly cyclic or alicyclic group with 5 – 8 carbons.

Compound 3 described in patent [214] has reportedly been used to inhibit the action of RNA-dependent RNA polymerase (RdRNP) *in vitro*, although no data are shown. In compound 3, X is S, O or NR₅ (R₅ being H or alkyl of 1 – 5 carbon atoms) R₁ is an unsubstituted or substituted heterocycle, a phenyl group, biphenyl or o-phenylalkenyl group or a substituted alkyl group (of 1 – 5 carbon atoms that may be straight or branched chain). Either R₃ or R₄ is H with the other being an unsubstituted or substituted phenyl group or (CH₃)₃COOH (n being an integer from 1 – 5).

Patent [215] describes nucleoside analogues 4a and 4b that inhibit recombinant hepatitis C NS5 RdRNP *in vitro* but no other data are presented. In nucleoside analogues 4a and 4b, R is H, -NR₃R₄ or OR₅ (where R₂ is H, C₆H₅ alkyl, C₆H₅ alkenyl, C₆H₅ alkynyl, C₆H₅ cycloalkyl; R₁ and R₅ are H, C₆H₅ alkyl, C₆H₅ alkenyl, C₆H₅ alkynyl). Z is H, OR₅ or NR₃R₄ (where R₂ is H, C₆H₅ alkyl, C₆H₅ alkenyl, C₆H₅ alkynyl, C₆H₅ cycloalkyl; R₁ and R₅ are H, C₆H₅ alkyl, C₆H₅ alkenyl, C₆H₅ alkynyl). Y is N or C-X (where X is H, halogen, C₆H₅ alkyl, C₆H₅ alkenyl, alkylnyl, CN, CF₃, N₃, NO₂, C₆H₅ aryl, C₆H₅ heteroaryl and COR₇ (where R₇ is H, OH, SH, C₆H₅ alkyl 6 aminoalkyl, C₆H₅ alkoxy and C₆H₅ thioalkyl). R₃ is H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C₆H₅ alkyl, alkenyl, C₆H₅ alkenyl, C₆H₅ aryl.

2.2.3 Helicase
Patent [216] describes the inhibition of viral helicase, from the *Flaviviridae*, *Poxviridae* and *Papovaviridae* families. Compound 5 and its derivatives showed *in vitro* inhibition of the unwinding function of viral helicase in these virus families.

In compound 5, R₁ contains up to 7 hydrogen atoms. T is a bond or linker group and the dashed line, when present, represents a double bond. X is O or NR₅ and n is 0 or 1. R₂ is =O or =S when the dashed line is not present.

Compound 5 inhibits viral helicase, either directly or indirectly, thereby preventing the unwinding function of helicase. Viral helicases and in particular the HCV NS3 helicase, contain a binding site for ATP, which is cleaved by the ATPase activity of helicase and a separate binding site for double-stranded polynucleotide, which is unwound by the helicase unwinding activity. The energy generated by ATP cleavage is required for the unwinding activity. Compound 5 and its derivatives, were assayed for their ability to inhibit ATP cleavage and inhibition of unwinding activity of helicase enzyme *in vitro*. Inhibition of unwinding of HCV NS3 4A helicase was assayed using 3'-tailed double-stranded RNA/RNA hybrid as substrate. Inhibition of ATPase activity of HCV NS3 4A helicase by the tested compounds was followed spectrophotometrically. The inhibition of the cytopathic effect was performed on BVDV-infected MDBK cells and plaque reduction assay and cytotoxicity assay were done on HSV-infected vero cells in the presence and absence of the testing compounds. There is, however, no reported data concerning dengue virus helicase activity inhibition by this compound.

2.2.4 Polyaminos that interfere with viral-target cell receptor binding
The identification of a heparan sulfate (HS) proteoglycan (PG) as a receptor for dengue virus on multiple cell types suggests that highly charged polyaminos might act as effective inhibitors of viral infectivity [75]. Heparin and HS are structurally related glycosaminoglycans that exhibit many of the same biological activities [119]. While heparin is widely used clinically, development of drugs based on HS has lagged due to limited availability [120]. Other natural polysaccharides, such as chitin [121],
pentosan [122] and dextran [123], have been sulfated and used as heparin analogues. These fully sulfated polysaccharides have anticoagulant activity [121-123] and antiviral activity [124] (e.g., dextran sulfate inhibits HIV binding to T lymphocytes but low oral bioavailability has precluded clinical use in [125]). Large synthetic polymers, such as poly(vinyl sulfate), poly(acetylene sulfonate) and poly(naphthalene sulfonate), are highly charged heparinoid polymers with substantial antiviral and anticoagulant activities [126,127] but clinical use is precluded by poor clearance and high toxicity.

US patent [217] describes the use of polymers, including Suramin, for the prevention of dengue virus infection in vitro. Small sulfonated and sulfated polymers, such as suramin, offer certain potential advantages over larger more complex molecules [129]. Suramin was the first heparin analogue and has been widely used clinically [128] as an antimicrobial [130-131]. This class of small molecules is likely to have improved bioavailability because of their reduced size and charge. They may include hydrophobic or hydrogen bonding sites in their structures that can enhance protein binding. Sulfate groups are present in the natural HS-PG ligand for dengue virus envelope protein and target cell binding has been demonstrated to be sulfation dependent. Sulfonates are structurally similar to sulfate groups, giving a nearly identical delocalisation of negative charges on the three oxygen atoms bound to sulfur [132] and have the added advantages of being easy to introduce synthetically and stable to catabolism. However, this stability leads to long half-lives that are often associated with in vivo toxicity, a particular problem with suramin [128]. Naphthalene sulfonates have also been investigated as antiviral agents [133,134] and show potent anti-HIV activity [135]. Even simple aliphatic disulfonates act as heparin mimetics [135]. The widespread availability of polysulfonated agents and their wide range of molecular weights (100 – 2500 KDa) and net charge (-2 to -20) suggest that these compounds should be examined for potential to inhibit dengue virus infection.

Chemically defined, homogeneous heparin and HS oligosaccharides have clear advantages over the currently used polydisperse microheterogeneous preparations (including LMW heparins) but disadvantages over the simpler synthetic analogues previously described [45]. It should be possible to prepare heparin oligosaccharides that are extremely selective agents, i.e., having antiviral activity but no anticoagulant activity, such as the decasaccharide that was successfully used to block dengue virus envelope protein cell-binding [75]. Although these oligosaccharides can be prepared from the parent glycosaminoglycan in a single enzymatic step [136] the purification of sufficient amounts of these oligosaccharides is difficult, precluding their use as agents to block dengue virus infectivity [137-139]. The use of high-throughput screening methods to study competitive inhibitors of viral binding to receptors [129,140] offers new possibilities for drug discovery.

2.2.5 Ribavirin and related compounds: antiviral synthetic guanosine analogues

Ribavirin is an antiviral synthetic guanosine analogue that competitively inhibits inosine monophosphate dehydrogenase (IMPDH). Ribavirin is clinically used to treat hepatitis C infection. Although ribavirin, 6, and its analogues have been demonstrated to be effective in inhibiting dengue virus in vitro, it is ineffective in vivo [213]. Ribavirin analogues, however, remain promising investigational agents for treatment of dengue virus infection.

IMPDH is essential for pyrimidine nucleotides synthesis. The de novo synthesis of guanosine nucleotides and thus, the activity of IMPDH, is particularly important in viral replication. Analogous to lymphocyte and tumour cell lines [118], the de novo synthesis, rather than the salvage pathway is critical in the process of viral replication. US Patents [218,219] describe novel IMPDH inhibitors but provide no data on antiviral activity.

Patent [220] describes the antiviral activity of triaizinoindole, compound 7. Antiviral activity was demonstrated against a pestivirus, bovine viral diarrhea virus, which is related to dengue virus. Triaizinoindole compounds 7 inhibit replication of BVDV, by a plaque reduction assay in MDBK cells, with low cytotoxicity demonstrated by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cytotoxicity assay [141] and good in vivo bioavailability. In compound 7, A is substituent selected from NR₄R₂ (where R₁ and R₂ could be H, straight or branched alkyl groups (C₁ – C₆), aryl or aralkyl groups or could be substituted heterocyclic group). Q is linking moiety. Rₚ, R₅, R₆ are H or alkyl (C₁ – C₆), phenyl or CO-OR. m is integer from 0 to 6; q and r are integers from 0 to 4. W, X, Y and Z could be H, alkyl, aryl, aralkyl, halogen, acyloxy, S-alkyl, SO₂-alkyl, SO₂-alkyl, NH₂SO₂, NO₂ or NH₂.

Replication of the viral genome can be inhibited by interferring with structure and/or the function of membrane-bounded viral replication complexes. In patent [221] the inhibitory action of benzimidazole compounds, on the cytotoxic effect in both BVDV and HCV-infected Madin Darby bovine kidney (MDBK) cultured cells are reported. These compounds direct their inhibitory action to membrane bound viral replication complexes. Low cellular toxicity recommends
their development as antiviral drugs in treatment of infection by other flavivirus members, including dengue virus.

2.2.6 Monoclonal antibodies

Patent [222] describes a mAb that neutralises dengue 2 virus without causing ADE, in an in vitro model. While these and similar mAbs may be useful in treating infection in vivo, evaluation will be required. Dengue virus infection usually causes a self-limiting febrile illness, for which administration of complex treatments, such as those involving mAbs, would not be appropriate. However, some cases are complicated by life-threatening DHF or dengue haemorrhagic shock syndrome and, in these cases, the direct administration of mAbs (whole antibodies or Fab₂ fragments) may be appropriate.

2.2.7 Other approaches

2.2.7.1 Synthetic oligonucleotides as antiviral tools

Antisense oligonucleotides represent a potential therapeutic approach for the treatment of dengue infection. Cell-free translation of mRNA can be inhibited by the binding of an oligonucleotide complementary to mRNA. Zamcnič et al. [142] showed that a 13-mer synthetic oligonucleotide that was complementary to a portion of the Rous sarcoma virus genome inhibited viral replication in infected chicken fibroblasts. These early indications that synthetic oligonucleotides could be used to inhibit virus propagation and neoplasia were followed by bioavailability testing of synthetic radiolabelled oligonucleotides in vivo using colorectal application in lab animal model [223]. This patent, however, does not relate to treating dengue virus infection, and without substantial improvement of in vivo delivery methods antisense therapy will probably not be clinically relevant in the foreseeable future.

2.2.7.2 Antiviral flavonoids and plant extracts

Sanchez et al. [143] analysed the effect on dengue viruses of different flavonoids extracted and identified from the plants Tephrosia madrensis, Tephrosia vaudiflora and Tephrosia crassifolia. The flavonoids glabranine, 8, and 7-O-methyl-glabranine showed significant inhibitory activity, in a dose-dependent manner, on dengue virus infection in Rhesus monkey kidney cells (LLC-MK2) using a plaque assay inhibition test. The in vivo virucidal effect of these compounds against dengue virus still needs further investigation.

Patent [224] describes Helioxanthin compounds 9a, 9b and 9c, derived from the Taissania cryomeris plant, that inhibit plaque formation of yellow fever virus, a related flavivirus, in BHK cells. No other data concerning inhibitory action on dengue virus by these compounds are presented.

In compound 9, A is H, OH or forms a 1,3-dioxolane group with B such that A and B are O and are bridged together by a -CH₂- (methylene) group; C is H, OH or forms a 1,3-dioxolane group with B such that B and C are O and are bridged together by a -CH₂- (methylene) group; D is OH or forms a 1,3-dioxolane group with either A or C; R is a C₁ to C₅ alkyl group, a benzylic group or a C₁ to C₅ acyl group, D and E (can be same or different) are CH₃, CH₂OH, CH₂OR, CHO, COOH, COONa⁺, CH₃COOR (R₁ is a C₁ to C₅ alkyl group) or a keto or methylene group. F and G are H or Br; I is H, OH or Br; and J and K (can be the same or different) are CH₃, CH₂OH, CH₂OR, CHO, COOH, COONa⁺ or 1,3-dioxolane group (J and K are O and are bridged together by a methylene group).

These types of antiviral natural products are in very early development and can not yet be considered as agents for the treatment of dengue virus infection.

2.2.7.3 Inhibition of viral replication at the stage of membrane association

Patent [225] describes a new class of potential antiviral agents based on inhibiting morphogenesis of viruses, which acquire their envelope by budding through host cell membranes. In this patent a tissue culture model was used to show that inhibition of glycosidases by compound 10 inhibited replication of BVDV in a plaque reduction assay. Glucosidase inhibitor, 10, affects the trimming of glycan chains, which are required for viral generation and release from infected cells.

Glycosidase inhibitor 10 is 1,5-dideoxy-1,5-imino-D-glucitol or a derivative containing N-alkyl, N-acyl, N-aroyl, N-aralkyl or O-acyl, where R₁ is H, alkyl, alkenyl, alkoxy, acyl, aralkyl, acroyl, aralkoxy, heterocyclic groups; and R₂, R₄ and R₅ (the same or different) are acyl or arroyl; and R₆ is hydrogen or an alkyl, alkenyl, acyl, arroyl or an alkyl group.

In patent [226] castanospermine compounds are used as glucose analogues for inhibition of glucose transport, glucosyltransferase activities and glucosidase activities. Their inhibitory activity against glycosidases has led to the evaluation of these compounds as antiviral agents. Castanospermines are effective
in inhibiting BVDV production in MDBK cells. The glucosidase inhibition prevents virus replication in infected cells and a similar approach might also be effective in the case of dengue virus infection. It should be noted that inhibition of an enzyme involved in normal cellular function, such as a glucosidase, is likely to be associated with a substantially higher risk of toxicity, compared with targeting a viral-specific metabolic pathway.

Compounds with long chain N-alkyl amino or imino or oxo-derivatives, 11, were studied as new antiviral drugs in patent [227]. Their antiviral drug effect was suggested by inhibition of ceramide glucosyl transferase and glucosidas. The inhibition of these two enzymes affects viral expression as well as morphogenesis in tissue cultures. The inhibitory activity was shown against one or more of pestiviruses or flaviviruses (e.g., BVDV). Virus infected Hep-G 2.2.15 cells were tested to antiviral effect using intracellular DNA analysis, endogenous polymerase assay and secretion of viral particles. BVDV-infected MDBK cells were used to test the antiviral effect by measuring plaque reduction assay, yield assay and secretion of infectious BVDV particles. The uptake of the radiolabeled inhibitors was demonstrated by measuring radioactivity in the supernatant of Hep G2.2.15 and MDBK cells previously incubated with the inhibitors. However, while these in vitro results for BVDV are promising, no data are presented on dengue virus nor are in vivo data presented.

In compound 11, R is a C9-C16 alkyl or an oxo-substituted derivative. R2 is hydrogen, R3 is carboxy or a C1-C4 alkoxycarbonyl or R3 and R4 are either -(X-C)Y2 or -(CX)Y3 where n is 3 or 4, X is hydrogen, hydroxyl, amino, carboxy or C1-C4 alkyl, C1-C4 alkyloxy, C1-C4 alkoxy, C1-C4 hydroxyl, C3-C6 acyloxy. Y is hydrogen, hydroxyl, amino, carboxy. R2 is hydrogen. R3 is hydrogen, hydroxyl, amino, substituted amino or carboxy, alkoxy, alkoxy, amino, hydroxyl, acyloxy or aroylxy. 2.2.7.4 inhibition of dihydroorotate dehydrogenase

Patent [228] describes 6-fluoro-2-(2′-fluoro-1,1′-biphenyl-4-yl)-3-methyl-4-quinoinecarboxylic acid sodium salt (brequi-nar, 12), a specific dihydroorotate dehydrogenase (DHOD) inhibitor, as a potential drug for treatment of dengue virus infection. Compound 12, was studied using VSV, Kunjin, yellow fever and dengue virus infected cultured human liver (HuH7) and monkey kidney (Vero) cells by assessing inhibition of viral cytopathic effect. Drug action can be potentiated by synergistic administration of interferons. DHOD (EC 1.3.3.1) is an enzyme involved in the de novo pyrimidine biosynthetic pathway. Inhibitors of DHOD have demonstrated activity against certain classes of RNA virus. Since DHOD is required for normal cellular function, inhibitors of this enzyme might result in significant toxicity.

In compound 12, A is H, halogen, perhaloalkoxy, amino, allyl, NO2, CN, SO2CH3, alkyl, alkoxy, cycloalkyl, cycloalkene, aryl, arloxy, perhaloalkyl; or two adjacent groups A on ring b (n = 1 or 2) form a naphthalene ring system or phenyl ring to which they are attached. R is cyclohexyl, phenoxy or benzoxy or a phenyl ring which may be with A or R and an adjacent group A on ring b from a naphthalene or phenanthrene ring system with phenyl ring to which they are attached. Y is CO-OM, CONHR' (R' is alkyl), SO2M (M is H, Li, Na, K, 0.5 Ca or H) and T is = N- or = C(Z)- where either Z is hydrogen, NH2, OH, C1-C8 alkyl, C3-C7 cycloalkyl, aryl and C1-C5 perhaloalkyl or Z is a bridging moiety attached to ortho position of ring b of the adjacent biphenyl group, thereby completing a ring.

US Patent [229] describes N-(phosphonoacetyl)-L-aspartic acid as a specific inhibitor of de novo pyrimidine biosynthesis. This compound functions by blocking L-aspartic acid transcarbamylase, the enzyme responsible for the condensation of L-aspartate with carbamyl phosphate in the first step of pyrimidine nucleotide biosynthesis. This inhibition results in depletion of pyrimidine nucleotides (UTR CTP), as well as nucleotide intermediates like UDP-GlcN and CMP-NeuNAc, which are essential for elongation of the oligosaccharide chains [146,147]. Thus, while N-(phosphonoacetyl)-L-aspartic acid acts primarily by inhibiting nucleotide biosynthesis, the effect on its intermediates is also reflected in its end products, including, carbohydrates, proteins and nucleic acids. Data are presented relating to many viruses. These show that replication of some flaviviruses is reduced, as measured in a plaque reduction assay. No data for dengue virus is presented.

2.2.7.5 Inhibition of apoptosis

Caspses are cysteine proteases that are important mediators of apoptosis. US patent application [230] describes a family of caspase inhibitors, one use is for the treatment of infectious diseases, including dengue virus infection. Dengue virus infection is characterised by apoptosis [116] and it is suggested that apoptotic killing of infected cells relates to the pathophysiology of infection.

3. Expert opinion

3.1 Vaccines

Most effort in vaccine development is being devoted to developing live attenuated combinations of all four dengue virus serotypes that can be used in a practical immunisation schedule to generate a neutralising and long-lasting immune
Patents related to dengue virus infection

response to all dengue viruses. As discussed above, the presence of multiple serotypes circulating in the same geographic regions and the concern that inducing antibodies to one serotype could result in more severe and potentially life-threatening infection by a different serotype, will prevent release of vaccines not effective for all four serotypes. The need to generate a safe, well-tolerated and effective tetravalent vaccine is a formidable task and it is not clear when this will be accomplished. Meanwhile, DNA-based vaccines offer many technical advantages for rapid manipulation of vaccine candidates but are a relatively new and unproven area for investigation.

3.2 Antiviral drugs

The targeting of specific viral proteins important in the virus life cycle, such as proteases and RNA polymerase, offers the best hope for developing effective agents for treatment of dengue infection. Agents directed at interfering with dengue virus binding to its receptors, such as polyanions or mAbs, offer a unique approach to prevent initial viral infection or to block the spread of virus to noninfected tissues. As molecular targets become better defined new approaches for the development of antiviral drugs useful for treating dengue infection is expected. Apoptosis is an important process in normal development, homeostasis and disease. Apoptosis occurs in cells infected by dengue virus and other microorganisms and it is possible that apoptosis contributes to the pathophysiology of disease. The role of apoptosis in dengue virus pathogenesis is a fertile area for investigation but at this time considering using caspase inhibitors to prevent apoptosis, as a potential dengue virus therapeutic, is entirely speculative.

3.3 Future prospects

Almost 100 years after dengue fever was first identified as being caused by a filterable virus [2], there is no treatment for dengue virus infection, no vaccine and attempts to reinstate previously successful programs to control dengue virus by larvicidal spraying of its mosquito vector have failed. Dengue virus is now considered to be pandemic in the developing world [16]. The small genome and simple structure of dengue virus belie the enormous public health burden it has imposed on the developing world, yet at the same time suggest it should be readily amenable to research aimed at understanding the mechanisms involved in virulence and pathogenicity. A better understanding of the virus and the disease it causes should lead to treatments based on rational antiviral and host-protective drug design for the small number of patients with complicated dengue virus infection and developing safe and effective vaccine candidates and methods to reduce the incidence of infection.

Bibliography

Papers of special note have been highlighted as either of interest (†) or of considerable interest (**) to readers.


13. Of historic interest.


15. Extraordinary clinical research defining mode of transmission and clinical spectrum of dengue virus infection.


* Study indicating dendritic cells are an important primary target cell in dengue virus infection.


* Demonstration that heparan sulfate glycosaminoglycan is one important target cell receptor for dengue virus.


- Review of vaccine candidates.


- Initial clinical trial data on immunogenicity of a potential tetravalent vaccine.


- Description of a virulence-attenuating deletion mutation to generate a potential vaccine candidate.


- A promising chimeric virus vaccine candidate made up by combining elements of a clinically stable and effective vaccine strain of yellow fever virus and dengue virus.


- Recent review of potential dengue virus vaccines.


- Cryo-electron microscopy study of the structure of dengue virus.


- X-ray crystallographic study of the structure of the ectodomain of tick-borne encephalitis virus envelope protein.


108. CLEAVES GR, DUBIN DT: Methylation status of intracellular dengue Type 2 40S RNA. Virolology (1979) 96(1):159-165.


- Structure of the dengue virus protease.


Patents related to dengue virus infection

116. CHARRIER JD: Caspase inhibitors and uses thereof. USP TO (2001).


Patents

Patents of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.


* Approach to generating vaccine candidate by mutating a 3' non-coding region stem-loop structure.
   - Promising chimeric-virus vaccine.
   - A good demonstration that polyanions were effective inhibitors of envelope protein binding and dengue virus infectivity.

Affiliation
Mohamad Warda¹, Rory M Marks² & Robert J Linhardt³
¹Author for correspondence
²Department of Biochemistry, Faculty of Veterinary Medicine, Cairo University, Egypt
³Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA
Tel: +1 734 936 3257; Fax: +1 734 763 2025; E-mail: rmarks@umich.edu
Division of Medicinal Chemistry, Department of Chemistry and Department of Chemical and Biochemical Engineering, University of Iowa, Iowa City, IA 52242, USA
Tel: +1 315 355 8834; Fax: +1 319 355 6634; E-mail: Robert-linhardt@uiowa.edu