Dengue

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Dengue viruses have spread rapidly within countries and across regions in the past few decades, resulting in an increased frequency of epidemics and severe dengue disease, hyperendemicity of multiple dengue virus serotypes in many tropical countries, and autochthonous transmission in Europe and the USA. Today, dengue is regarded as the most prevalent and rapidly spreading mosquito-borne viral disease of human beings. Importantly, the past decade has also seen an upsurge in research on dengue virology, pathogenesis, and immunology and in development of antivirals, vaccines, and new vector-control strategies that can positively impact dengue control and prevention.

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Introduction

Dengue is an arthropod-borne viral disease caused by the four dengue virus serotypes (DENV 1–4), which are transmitted by *Aedes* mosquitoes. Dengue has evolved from a sporadic disease to a major public health problem with substantial social and economic effect because of increased geographical extension, number of cases, and disease severity.

Dengue is endemic in more than 100 countries in southeast Asia, the Americas, the western Pacific, Africa and the eastern Mediterranean regions (figure 1), and its incidence has increased 30-fold in the past 50 years.[4] Recent estimates made in 2013 cite that 390 million people have dengue virus infections with 96 million cases annually worldwide, more than three times WHO's 2012 estimate.[1] However, the true disease burden is not well known, especially in India, Indonesia, Brazil, China, and Africa.[1] Prospective cohort studies[5,6] in Nicaragua and Thailand indicate an incidence of dengue virus infection of 6–29% per year. Other studies[7,8] calculate that 2–28-fold more dengue cases occur than are reported by national surveillance systems and support use of expansion factors for estimations.

Dengue activity in Africa has increased substantially, although lack of clinical suspicion and diagnostic tests probably underestimated dengue prevalence in the past.[9] Dengue outbreaks in India and the eastern Mediterranean region have progressively increased, with recent reports of cases in Pakistan, Saudi Arabia, Sudan, Yemen, and Madagascar; cases of dengue haemorrhagic fever/dengue shock syndrome, and circulation of several serotypes have also been reported.[9] Resurgent dengue activity has been documented in Hawaii, the Galapagos islands, Easter Island, Hong Kong, and Buenos Aires. Dengue introductions have also been reported in Florida, southeastern France, and Madeira island.[9,10] The presence of *Aedes albopictus* and *Aedes aegypti* mosquitoes in Europe, together with increasing travel and pathogen introduction, poses a risk for transmission.[11] Increasingly, co-infections of dengue occurring with leptospirosis, malaria, HIV/AIDS, and chikungunya are reported, as well as potential dengue transmission by blood transfusion.[12] Lastly, travellers play an important role in global dengue epidemiology, carrying viruses from one region to another.[9,11]

Dengue exacts a high economic burden on both governments and individuals. Dengue illness in the Americas costs US$2·1 billion per year on average, excluding vector control, exceeding costs of other viral illnesses.[7] In southeast Asia, 2·9 million dengue episodes and 5906 deaths were estimated annually, with an annual economic burden of $950 million.[13] Its rapid global emergence is related to demographic and societal changes of the past 50–60 years, including unprecedented population growth, increasing movement of people (and consequently viruses), uncontrolled urbanisation, climate change, and breakdown in public health infrastructure and vector control programmes.

Transmission dynamics

Dengue transmission results from interactions between people, mosquitoes, viruses, and environmental factors. Local human movement is a spatiotemporal driver of transmission dynamics important for dengue virus amplification and spread.[14] House-to-house human movements define spatial patterns of dengue incidence, causing marked heterogeneity in transmission rates.[14] Fine-scale spatiotemporal clustering of dengue transmission exists, with houses with high dengue virus transmission risk contributing disproportionately to virus amplification and spread.[15]

The implications of inapparent dengue virus infection in dengue transmission, disease pathogenesis, and vaccine assessment needs careful consideration. Viral characteristics, the host's immune and genetic background, and epidemiological factors lead to variable ratios of symptomatic to inapparent infections.[5,6,16,17] Inapparent infections and under-reporting of cases should be considered in estimation of the disease and economic burden.[8]

Search strategy and selection criteria

We searched PubMed for articles pertaining to dengue and each of the topics discussed in the Review. Search terms included "dengue" and "epidemiology", "modeling", "phylogenetic", "clinical", "diagnosis", "vaccine", "antiviral", "pathogenesis", "immunopathogenesis", "innate immunity", "antibody", "T cell", and "vector control", among others. The most relevant and recently published references were then selected to comply with the reference number limitation.

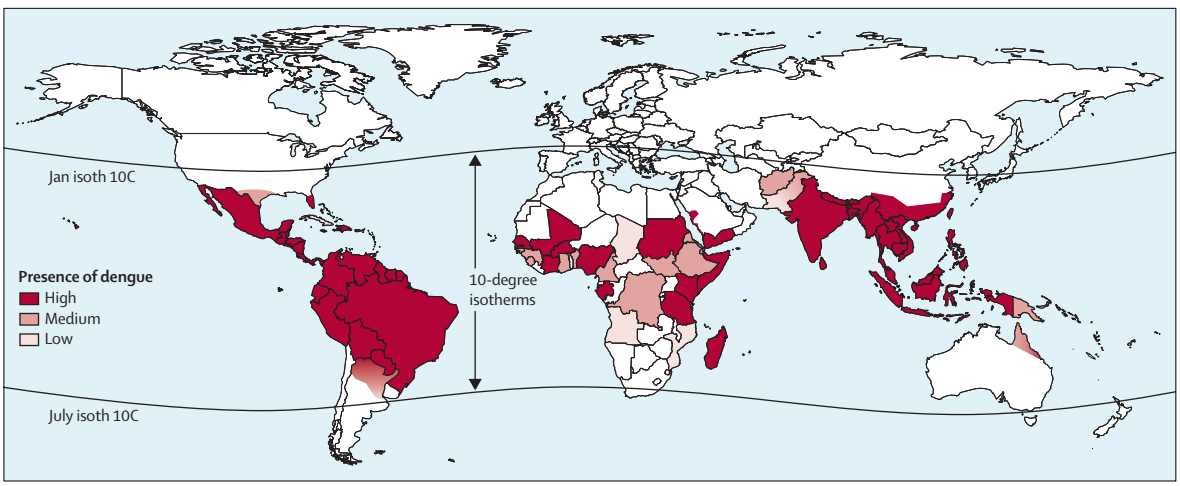

***Figure 1*: Global dengue burden, 2014**
Data from Bhatt and colleagues,[1] Healthmap,[2] and WHO[3] were integrated to indicate the relative amount of dengue globally according to best estimates.

The use of mathematical models to help understand multiple aspects of dengue transmission has greatly increased. Some models define patterns of spatiotemporal dependence consistent with the expected effects of homotypic and heterotypic immunity and immune enhancement of disease.[18] Other models suggest that the shift of dengue cases in Thailand towards older age groups is attributable to a shift in the age distribution of the population and its effect on the force of infection.[19] By contrast, the shift of dengue haemorrhagic fever cases to children in Brazil is explained by an accumulation of multitypic immunity in adults, with reduced probability of remaining susceptible to infection and decreased mean age of secondary infection.[20] These factors should be considered in the design of prevention strategies.

Virus evolution and fitness

The four dengue virus serotypes are genetically diverse and share limited identity (around 60–75%) at the aminoacid level. Viruses within the same serotype have about 3% difference at the aminoacid level and 6% difference at the nucleotide level and are phylogenetically divided into genotypes and clades. Genetic variations between serotypes and clades are important determinants of differential viral fitness, virulence, and epidemic potential.[21–24] For example, strains with a replicative advantage in both humans and mosquitoes can spread more rapidly and successfully than can strains with lower replicative abilities, and might eventually displace strains with lower fitness.[21–24] Viral genetics also influence interactions of the virus with the host's pre-existing immune response,[24] as well as the overall efficacy of host anti-viral immune responses. Consequently, particular serotypes and clades have been associated with differential clinical manifestations and disease severity.[24,25] In addition, the population structure of dengue virus genomes within an individual during acute disease (ie, intrahost diversity) could have a role in determination of disease outcome. With the use of deep sequencing technologies, the study of intrahost diversity is actively evolving, and recent reports suggest that the extent of dengue virus diversity during acute infection is lower[26] than previous estimates suggest.[27] The association between intrahost diversity and disease outcome is an area of active investigation.

In terms of serotype and strain introductions, studies in Iquitos, Peru, suggest that the establishment of a new serotype requires a period during which environmental conditions are favourable for virus amplification, with three phases: amplification, replacement, and epidemic transmission.[28] Substantial genetic diversity among circulating viruses indicates that dengue virus is frequently introduced into both semiurban and rural areas from other populations.[29] Accordingly, invasion and establishment of viruses from outside of an area reduces the extent of lineage persistence. Lastly, the implications of sylvatic human infections also deserve careful study.[30]

New dengue case classification

After an incubation period of 4–8 days, infection by any dengue virus can produce a wide spectrum of illness, with most infections asymptomatic or subclinical. Most patients recover after a self-limiting (although debilitating) illness, while a small proportion progress to severe disease, mostly characterised by plasma leakage with or without bleeding. Illness begins abruptly, followed by three phases: febrile, critical, and recovery. The critical period occurs around defervescence, when an increase in capillary permeability accompanied by increased haematocrit can occur, leading to hypovolaemic shock that can result in organ impairment, metabolic acidosis, disseminated intravascular coagulation, and severe haemorrhage. Severe dengue also includes patients with hepatitis, neurological disorders, myocarditis or severe bleeding without plasma leakage or shock. If untreated, mortality can be as high as 20%, whereas appropriate case management and intravenous rehydration

can reduce mortality to less than 1%.[9] Persistent symptoms (eg, arthralgia or fatigue) in adult dengue patients up to 2 years after illness have been reported in 57% of studied patients.[31] The implications of this phenomenon deserve additional study.

A revised WHO case classification was introduced in 2009, replacing the traditional dengue fever and dengue haemorrhagic fever/dengue shock syndrome with dengue with and without warning signs and severe dengue (appendix).[9] The revised guidelines seek to improve triage and appropriate treatment,[9,32–34] because early recognition of warning signs should alert clinicians as to patient prognosis and enable correct triage and management decisions. Several study results show increased sensitivity for identification of severe cases with the revised classification.[32,33] However, some think that the new system could reduce the emphasis on the plasma leakage syndrome, increase the burden for resource-poor dengue-endemic countries, and inflate the number of cases.[35]

Dengue diagnosis

Diagnosis is important for clinical management, surveillance, and research. Diagnostic options include assays to detect the virus or its components (genome and antigen) or the host response to the virus. Assay choice depends on the timing of sample collection and the purpose of testing (appendix).[9] Viraemia is detectable for roughly 4–5 days after fever onset and correlates closely with fever duration. In a primary infection, anti-dengue-virus IgG evolves relatively slowly, with low titres 8–10 days after fever onset, whereas anti-dengue-virus IgM is detected typically about 5 days after fever onset and lasts 2–3 months. In secondary infections, anti-dengue-virus IgG evolves rapidly, with high titres soon after fever onset. In some cases, anti-dengue-virus IgM can be undetectable.[9]

Serum is the sample of choice, although plasma, blood, and tissues (liver, spleen, lymph nodes, lung, and brain collected from fatal cases) are also useful. The *A albopictus* C6/36 mosquito cell line is the preferred virus isolation system for routine diagnosis, although mosquito inoculation is the most sensitive method. Immunofluorescence assays with serotype-specific monoclonal antibodies (MAbs) or reverse transcriptase (RT)-PCR are employed for serotype identification. RT-PCR and real-time RT-PCR have become the methods of choice for genome detection. Viral RNA can be extracted from serum, blood, plasma, tissues (including formalin-fixed specimens), blood collected on filter paper, and (more recently) saliva. Many RT-PCR and real-time RT-PCR protocols are available, although few have been carefully validated. A recent Centers for Disease Control and Prevention (CDC) RT-PCR assay has been produced that enables dengue diagnosis in the first 7 days of illness.[36] Protocols for RT-PCR and real-time RT-PCR for multiplex detection of several arboviruses and haemorrhagic fever viruses are also available.[37] Secretion of viral non-structural (NS)1 protein from dengue-virus-infected cells offers a window of opportunity for early diagnosis, because NS1 can be detected in the blood up to 9 days after fever onset and in tissue samples.[38] Commercial rapid tests and ELISA kits are available, yielding sensitivities ranging from 54 to 93%, with less sensitivity in secondary infections.[39]

Detection of anti-dengue-virus IgM, which reveals an active or recent infection, is the most widely used test in laboratory surveillance. Different ELISA formats detect anti-dengue-virus IgM with different degrees of sensitivity and specificity.[40] Detection of IgA and IgE in serum, saliva, and urine have been proposed as diagnostic alternatives.[41] The haemagglutination inhibition assay, IgG ELISA, and neutralisation assays are useful for detection of previous exposure (appendix). The neutralisation assay is the most specific assay for measurement of anti-dengue-virus antibodies. The plaque reduction neutralisation test (PRNT) has been widely used in seroepidemiological surveys and vaccine studies; however, reproducibility between laboratories is low.[42] Numerous factors contribute to PRNT heterogeneity, including cell line, expression of receptors and attachment factors, complement, virus propagation cell line and resulting maturation state of the virion, temperature, and time of incubation.[43,44] Several alternative microneutralisation, immunospot and flow cytometry-based neutralisation assays are in use. Accessible reference reagents, proficiency testing and algorithms to adjust for protocol differences should be implemented to improve quality assurance among neutralisation assays.[42] Future diagnostic methodologies include microsphere-based immunoassays, nano-diagnostic and immunosensors, microarray technology to simultaneously screen samples for many different viruses, and biosensor technology for rapid discrimination of biological components in complex mixtures.[45]

See Online for appendix

Several diagnostic and prognostic assays are becoming available to identify severe cases in the early stages of illness.[9] Increased viraemia and NS1 levels have been associated with disease severity,[46] although further assessment is needed (appendix). Ultrasonography serves as a useful aid in prediction of dengue severity.[47] Microarray analysis of dengue cases enables identification of genes that are differentially regulated among patients with different disease severity.[48,49] Recently, high-mobility-group box 1 protein (HMGB1) was proposed as an auxiliary biomarker for early diagnosis,[50] monocyte chemotactic protein 1 (MCP-1) was increased in patients with warning signs,[51] and overexpression of leucine-rich glycoprotein 1, vitamin D binding-protein, and ferritin was found in plasma from patients with severe disease.[52] Although these biomarkers are not yet validated or available, promising candidates are emerging.

Dengue virus and the immune response

Dengue virus enters target host cells via clathrin-dependent receptor-mediated endocytosis.[52] Numerous putative receptors have been identified on human and mosquito cells, while dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) serves as a dengue virus attachment factor on dendritic cells.[54] In secondary infections, pre-existing antibodies bind to DENV virions and enable Fcγ receptor-mediated uptake by target Fcγ receptor-bearing cells, a process known as antibody-dependent enhancement. After endocytosis, a pH-dependent conformational change allows escape of viral RNA from the endosome, followed by translation in the endoplasmic reticulum and replication in invaginated membrane vesicles.[55] After association of the viral RNA with the capsid protein and budding into the endoplasmic reticulum to acquire a lipid membrane coated with membrane (prM/M) proteins and envelope (E) proteins, the virion exits through the host secretory pathway (figure 2).

Virus maturation

Cleavage of prM/M from the virion as it exits the cell is required for generation of mature DENV virions, which have a smooth marble-like structure, whereas immature or partially immature virions have a spiky appearance (figure 2).[56] Because of differential exposure and conformation of E and prM/M proteins on the surface of mature versus immature virions, the maturation state of flaviviruses modulates both the cell types that are infectable (owing to specific receptors expressed) and the interaction of the virion with particular antibodies.[56,57] However, the maturation state of DENV virions in human beings is currently unknown. During propagation in vitro, both mature and immature virions are produced, although the relative amounts vary substantially by cell type.[56] Another conceptual advance is that DENV virions are not static but rather dynamic, breathing structures, thus enabling antibodies with cryptic epitopes to bind and exposing the membrane underneath the layer of viral E and prM/M proteins.[44,57] Thus, cell type, temperature, and time of virus-antibody incubation can strikingly alter neutralising antibody titres.[44]

Hijacking of the host cell machinery by dengue virus

Dengue virus uses several mechanisms to hijack host cell machinery to facilitate viral replication (figure 2). Dengue virus translation and replication occur in the endoplasmic reticulum of host cells, which undergoes rearrangement and expansion during infection. Although this initial rearrangement is independent of the unfolded protein response (UPR),[58] dengue virus manipulates the UPR to cope with endoplasmic reticulum stress throughout infection.[59] Particular non-structural proteins (NS4A, NS2B/3) induce the UPR to reduce host cell death during viral replication.[60] Additionally, dengue virus induces autophagy and regulates lipid metabolism to enhance replication,[61] and a functional autophagy pathway is necessary for virus maturation and production of infectious virions.[62]

The innate immune response and viral evasion strategies

Pattern recognition receptors such as Toll-like receptors (TLRs) and intracellular sensors such as the helicases melanoma differentiation-associated protein 5 (MDA5) and retinoic acid-inducible gene 1 (RIG-I) are often one of the first lines of defence in the innate immune response recognising viral RNA. Human TLR3 recognises dengue virus infection after endosomal acidification and induces strong interferon α/β responses in vitro,[63] whereas stimulation of TLR3, 7, and 8 in monkeys during dengue virus infection is protective.[64] Both RIG-I and MDA5 are induced during dengue virus infection and are involved in interferon β induction.[63] Infection of Fcγ receptor-bearing cells by dengue virus complexed to non-neutralising antibodies during antibody-dependent enhancement results in down-regulation of TLR3, 4, and 7 and TLR signalling, as well as disruption of RIG-I and MDA5 signalling cascades, leading to suppression of interferon α/β-mediated antiviral responses.[65]

Dengue virus can interfere with RNA interference (RNAi) pathways via two distinct mechanisms. Dengue virus infection results in production of a subgenomic flavivirus RNA (sfRNA) from the 3'-untranslated region of the genome that can inhibit cleavage of double-stranded RNA (dsRNA) by the dicer enzyme.[66] Dengue virus infection is also able to suppress the RNAi pathway by expression of NS4B.[67]

Interferon α/β is a powerful inhibitor of dengue virus infection; hence, dengue virus has developed strategies to interfere with interferon α/β pathways. Dengue virus NS2B/3 protease directly cleaves the human mediator of interferon regulatory factor 3 activator (MITA or STING) within the interferon induction pathway to downregulate antiviral responses triggered upon dengue virus infection.[68] Cells respond in an autocrine and paracrine manner to interferon released from infected cells, and signalling through the interferon α/β receptor is mediated via the STAT1/2 signalling pathway. Dengue virus can also interfere with this signalling pathway. NS2A, NS4A, and NS4B associate with cellular membranes and when expressed together can inhibit STAT1 phosphorylation in host cells.[69] Dengue virus NS5 bound to the host protein UBR-4 interacts with STAT2 and mediates STAT2 degradation via the proteosome.[70]

Adaptive immune response

After primary dengue virus infection in humans, most of the neutralising antibody response is directed to virion-specific epitopes that are not present on recombinant E monomers,[71] and dominant epitopes responsible for highly potent, serotype-specific humoral immunity seem to be located in the hinge region of E,

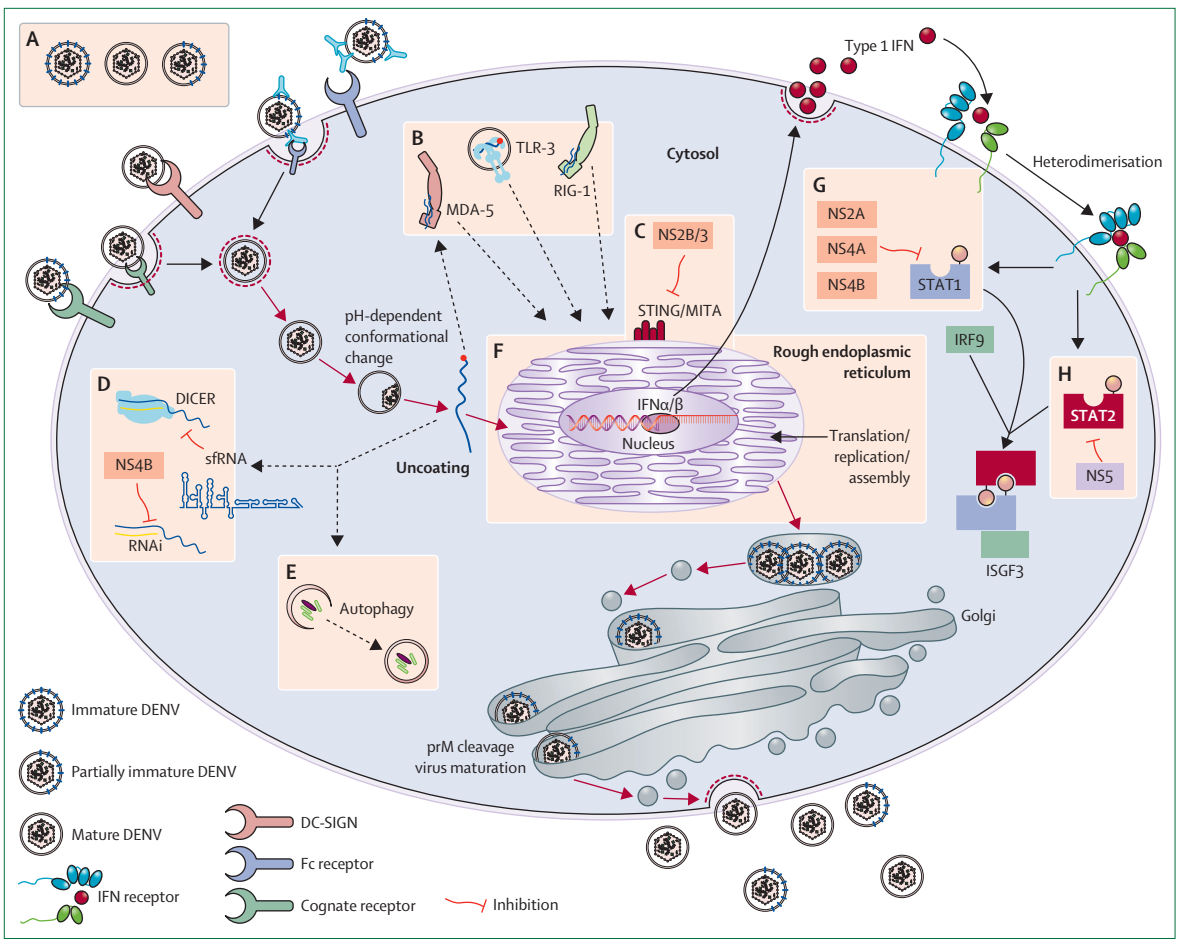

***Figure 2*: Dengue virus lifecycle and subversion of the cellular antiviral response**
Red arrows show the dengue virus lifecycle. Subversion of the cellular antiviral response is indicated with tan squares labelled with letters (A–H). (A) Virus maturity can affect infectivity and antibody binding and neutralisation. (B) During antibody-dependent enhancement, infection can disrupt Toll-like receptor, RIG-I, and MDA5 signalling cascades, leading to suppression of interferon α/β-mediated antiviral responses. (C) Dengue virus non-structural protein 2B/3 protease cleaves MITA (or STING) within the interferon induction pathway to downregulate antiviral responses. (D) Dengue virus subgenomic flavivirus RNA and NS4B can interfere with the RNAi response. (E) Dengue virus-induced autophagy might enhance viral replication. (F) Dengue virus, in particular NS4A, induces endoplasmic reticulum hypertrophy and manipulates the unfolded protein response to cope with endoplasmic reticulum stress throughout infection. (G) NS2A, NS4A, and NS4B when expressed together can inhibit STAT1 phosphorylation and translocation to the nucleus. (H) Dengue virus NS5 interferes with interferon α/β signalling by mediating STAT2 degradation via the proteasome. DENV=dengue virus. sfRNA=subgenomic flavivirus RNA. TLR=Toll-like receptor.

including quaternary epitopes that span adjacent E dimers.[71,72] However, most human anti-dengue-virus antibodies seem to be serotype cross-reactive, with a large proportion directed to the prM/M protein and the fusion loop of the E protein.[73,74] A massive dengue-virus-specific plasmablast response occurs during the acute phase of secondary infection,[75] with a high degree of serotype cross-reactivity.[76] With respect to T cells, in addition to their potential role in dengue pathogenesis,[77] a protective role has recently been proposed for CD8-positive T cells.[78]

Dengue pathogenesis

The pathophysiological basis for severe dengue is multifactorial. Protective versus pathological outcome depends on the balance among the host genetic and immunological background and viral factors (figure 3).[77]

Host risk factors for dengue haemorrhagic fever/dengue shock syndrome

Primary dengue virus infection provides lifelong protection against the infecting serotype and transient cross-protection against heterologous serotypes. Epidemiological studies suggest that dengue haemorrhagic fever/dengue shock syndrome occurs mostly in individuals during secondary dengue virus infection with a different serotype and in infants with a primary infection born to dengue-immune mothers.[79] Viral genetics, serotype sequence, and time interval between infections can modulate secondary infection outcome. Sequence of infection can also affect the magnitude of the T cell response in secondary infections.[80] Furthermore, an increased interval between infections is associated with high risk of disease severity and increased case fatality rate.[81] Likewise, a longer interval

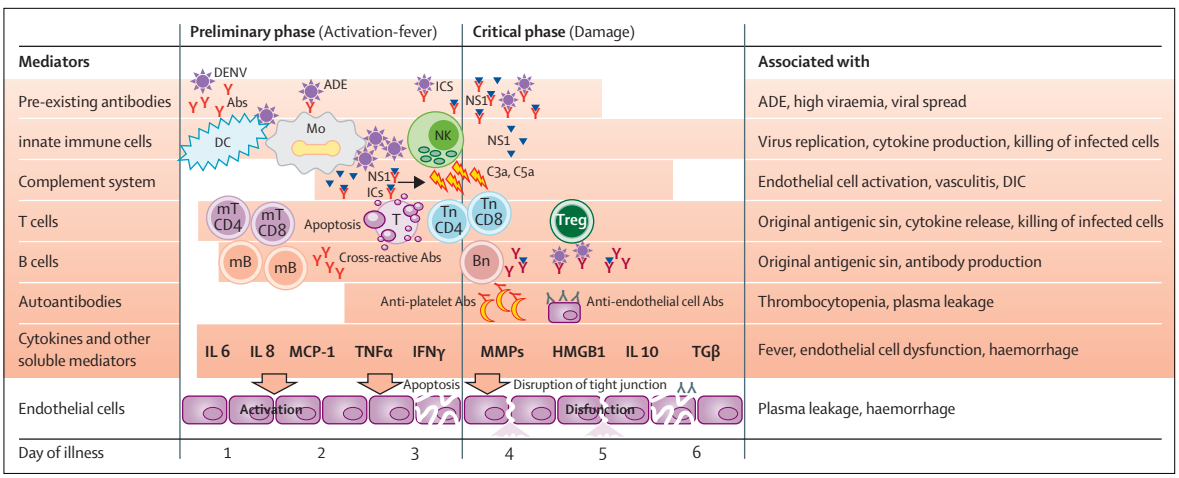

***Figure 3*: Pathogenesis of dengue virus infection according to phase of illness**
Possible mechanisms include the presence of preexisting antibodies that might mediate ADE; infection and activation of innate immune cells; activation of the complement system, T cells and B cells; and the production of auto-antibodies. Cytokines and soluble mediators, sequentially released by different cells as a consequence of immune activation, play an important part in disease pathogenesis by mediating plasma leakage through endothelial cells. Increased immune activation is associated with the severity of dengue illness. The increased levels of some proinflammatory cytokines that act on the vascular endothelium do not necessarily coincide chronologically with plasma leakage, as immune activation precedes defervescence and the onset of plasma leakage. DENV=dengue virus. Abs=antibodies. ADE=antibody-dependent enhancement. Ics=immunocomplexes. DC=dendritic cells. Mo=monocytes/macrrophages. NS1=NS1 protein. mT=memory T cell. Tn=naive T cell. Treg=regulatory T cell. mB=memory B cell. Bn=naive B cell. DIC=disseminated intravascular coagulation.

between sequential dengue virus infections has been associated with symptomatic as opposed to inapparent infection outcome.[17,82] To what extent tertiary and quaternary dengue virus infections contribute to severe illness is not clear; however, studies in hospitalised patients and seroepidemiological surveys suggest that it is low.[83] A 2013 study in Iquitos supports that postsecondary infections reduce the risk of illness.[84]

Bronchial asthma, diabetes, sickle-cell anaemia, particular ethnicities, and other host genetic characteristics have been associated with severe disease. HLA and non-HLA genetic factors (eg, vitamin D receptor, Fcγ receptor IIA, G6PD deficiency, tumor necrosis factor [TNF] α, interleukin 10) have been associated with disease severity.[85,86] Furthermore, alleles of *MICA* and *MICB* associated with symptomatic versus asymptomatic infection and *MICB* and *PLCE1* alleles associated with susceptibility to dengue shock syndrome have been identified.[87,88] Reduced severity of dengue disease in black individuals as compared with white individuals has been observed.[89] Finally, an increased rate of hospital admission and case fatality of dengue haemorrhagic fever/dengue shock syndrome in children versus adults during secondary infection has been reported;[90] differences in baseline microvascular permeability between children and adults could contribute to this phenomenon.[91]

Antibody-dependent enhancement

Early studies suggested a role for antibody-dependent enhancement in dengue pathogenesis; [92] however, not all studies support this hypothesis.[93] The following observations suggest that antibody-dependent enhancement can occur in vivo: undiluted sera obtained early from patients with secondary infection enhanced dengue virus infection in vitro,[94] infants born from dengue-immune mothers had higher viral burden than infants born to dengue non-immune mothers and had immune activation associated with disease severity, [95] and lethal antibody-dependent enhancement has been shown in dengue mouse models.[96] Virus-antibody complexes bind to Fcγ receptor-bearing cells, resulting in increased infected cell mass and a rise in viraemia.[65] Models suggest that at the population level, antibody-dependent enhancement can provide a competitive advantage to dengue virus serotypes that undergo enhancement compared with those that do not, conferring a fitness advantage with a natural selection for the former strains.[97]

T-cell response

During a primary infection, both serotype-specific and cross-reactive memory T-cell responses are produced. The expression of viral epitopes on infected cells during a secondary dengue virus infection triggers activation of serotype-cross-reactive memory T cells, with the production of pro-inflammatory cytokines ultimately resulting in plasma leakage in the vascular endothelium. Activation of memory T cells with low affinity for the present infecting virus but high affinity for previous infecting serotype(s) has been reported,[98] and the level of T-cell activation has been shown to correlate with disease severity.[77] Viral clearance mechanisms are suboptimal because the low-affinity T cells are less able to eliminate infected cells.[77] Data from Vietnamese children suggest that T-cell activation in blood is not synchronous with

commencement of capillary leakage, and the possible sequestration of activated T cells in tissues has been suggested.[99] Furthermore, the ratio of regulatory T cells to effector T-cell responses was increased in patients with mild compared with severe disease.[100] In addition, a regulatory immune pattern in homologous versus a pro-inflammatory pattern in heterologous dengue virus secondary infection has been reported.[101]

Cytokine storm and vascular leakage

Cytokine production is observed in patients with dengue haemorrhagic fever/dengue shock syndrome, changing rapidly over the course of illness. Direct viral infection of endothelial cells does not seem to be the major cause of plasma leakage;[65] rather, several soluble factors, produced by T cells, monocytes, macrophages, and mast cells, have been proposed to increase vascular permeability in primary endothelial cells, including TNFα, interleukin 6, interleukin 8, interleukin 10, interleukin 12, macrophage migration inhibitory factor, HMGB1, MCP-1, and matrix metalloproteinases.[77,102–104] Endothelial permeability can also be affected by the maturation state of NS4B, which modulates the cytokine response in monocytic cell lines.[105] In addition, secreted NS1 protein, together with anti-NS1 antibodies and complement activation, might be involved in dengue-virus-induced vascular leakage.[106] Finally, a role for the endothelial surface glycocalyx in regulating fluid flow across the microvasculature has been proposed.[107] The mechanisms underlying endothelial dysfunction are still not well understood.

Complement activation

Around defervescence, when plasma leakage is apparent, high levels of complement activation products C3a and C5a are detected in plasma, followed by accelerated consumption and large reduction of complement components in patients with dengue shock syndrome.[108] NS1 is an important trigger for complement activation via binding of antibodies to NS1 expressed on infected cells.[106] Also, NS1 released from dengue-virus-infected cells can directly modulate complement factors.[109] Activation of the complement system can stimulate production of inflammatory cytokines associated with DHF/DSS and trigger local and systemic effects implicated in intravascular coagulation.[108]

Autoimmunity

Although controversial, autoantibodies resulting in platelet and endothelial cell dysfunction might be involved in dengue haemorrhagic fever/dengue shock syndrome pathogenesis. Antibodies to some E protein epitopes can bind to human plasminogen and inhibit plasmin activity.[110] Anti-NS1 antibodies correlate with disease severity, and cross-reaction of anti-NS1 antibodies with liver and endothelial cells and platelets has been proposed to trigger these cells to express nitric oxide and undergo apoptosis.[110,111]

Interventions

Vaccines

Concern about antibody-dependent enhancement and its role in dengue haemorrhagic fever/dengue shock syndrome supports the necessity for tetravalent dengue vaccines that stimulate balanced immune responses to the four serotypes (panel). However, development of multivalent dengue vaccines has been hampered by difficulties in induction of a balanced immune response. Live attenuated and inactivated viruses, recombinant proteins, and DNA vaccines are under development as vaccine candidates (table 1).[112]

The first proof-of-concept dengue vaccine efficacy trial[114] showed a low efficacy for DENV 2, raising several important issues. First, the discrepancy between the chosen immune correlate (PRNT) and the trial results suggest that this neutralisation assay might not be sufficiently predictive of dengue virus infection outcome. Alternatively, the increased neutralising antibody titres could have been insufficient to protect against the particular DENV 2 epidemic strain, or the clinical attack rate of the circulating strain could have been unusually high. Other immune correlates of protection could be crucial. Recent evidence in human populations[78] and mouse models[114] suggests a protective role for CD8-positive T cells, with most epitopes located in non-structural

Panel: **Characteristics of an ideal dengue vaccine and challenges to its development**

Characteristics

- Safe in children and adults
- Avoids ADE and pathogenesis
- Requires only one or two doses
- Induces a balance between reactogenicity and immunogenicity
- Genetically stable
- Stimulates neutralising antibodies and Th1 cell-mediated immunity
- Induces long-lasting immunity and protection
- Easily stored and transported
- Cost effective

Challenges

- Possibility exists of triggering ADE
- Vaccine must be tetravalent
- Dengue virus serotypes do not induce long-lasting heterotypic immunity
- No ideal animal model exists for immunisation studies
- Markers of viral virulence are not well established
- Correlates of protection are not well defined
- Subsequent infection (especially after a long time interval) may lead to severe dengue
- Vaccine candidates should be evaluated in geographic areas with different transmission patterns

ADE=antibody-dependent enhancement.

	Description	Clinical trial status
Chimeric live-attenuated vaccine		
YF17D/dengue chimeric vaccine	Recombinant infectious cDNA clone of yellow fever 17D vaccine strain as a backbone, substituting prM and E protein genes with those of the four dengue virus serotypes	Phase 3
Live-attenuated vaccine		
Tetravalent live attenuated virus	Attenuation by serial passage in PDK cells	Phase 1/2
Dengue virus infectious clone live-attenuated vaccine		
Chimeric recombinant attenuated vaccine	Attenuated DENV 2 infectious clone containing prM/M and E of DENV 1, DENV 3, and DENV 4	Phase 2
3′-UTR deletion mutant attenuated vaccine	Attenuating deletion of a 30 nucleotide sequence in 3′-UTR of DENV 1, DENV 3 and DENV 4; production of chimera with DENV 2 prM/M and E in a DENV 4-attenuated backbone	Phase 1/2
DNA		
D1ME	prM and E protein genes	Phase 1
Protein		
r80E	Expression of N-terminal 80% E protein in insect cells	Phase 1
cEDIII	Domain III of E protein gene fused to p64K protein of *Neisseria meningitides* and expressed in *Escherichia coli*	Preclinical
Inactivated		
Purified inactivated dengue virus	Whole purified inactivated virus	Phase 1
Virus-like particle		
EDIII-capsid protein	Chimeric protein comprising domain III of E protein and the capsid protein of DENV 1–4	Preclinical
Virus vector		
Alphavirus VRP	Alphavirus replicon particles expressing two configurations of dengue virus E antigen (subviral particles [prME] and soluble E dimers [E85])	Preclinical
Adenovirus	Tetravalent formulation combining two bivalent adenovirus constructs	Preclinical
Measles virus	Expression of dengue virus antigen by a vector derived from live-attenuated measles vaccine	Preclinical

PDK=primary dog kidney. DENV=dengue virus. 3′-UTR=3′-untranslated region.

Table 1: **Candidate dengue vaccines**

genes;[78] because the backbone of the Sanofi Pasteur chimeric vaccine was the 17D yellow fever virus, it thus lacks T-cell responses to dengue virus non-structural proteins. The ongoing large phase 3 trial will be key to establishment of whether the vaccine confers protection against clinical disease or not.

For the **phase 3 trial** see http://www.bioinformatics.org/dengueDTDB/Pages/m.htm

Another avenue being pursued by several vaccine developers is the dengue human infection model, consisting of experimental dengue virus challenge after vaccination in a small number of volunteers to accelerate identification of vaccine candidates for phase 2b/3 efficacy trials, investigate correlates of protection, and assess the longevity of vaccine responses.[115] A crucial need exists to better understand immune responses to both natural infections and vaccine candidates and to identify robust correlates of protection, including investigation of optimised neutralisation assays, the quality of neutralising antibody responses, antibody avidity, antibody-dependent cell-mediated cytotoxicity, B-cell magnitude and breadth, and the magnitude, frequency, and multifunctionality of CD4-positive and CD8-positive T-cell responses.

Antivirals

At present, the US Food and Drug Administration (FDA) has not approved any drugs against dengue, but substantial efforts are underway to develop antiviral compounds that target viral or host factors[116] (table 2). Advances in acute-phase diagnostic assays make early diagnosis and treatment a more feasible scenario.

Viral factors

The virus entry step is an attractive antiviral target, and various strategies (table 2) have been deployed effectively to reduce infection in vitro. Another approach involves MAbs that neutralise dengue virus infection but are genetically modified to eliminate the capacity for enhancement, and such human and mouse MAbs are therapeutically effective in both high-dose and ADE mouse models of lethal dengue disease.[117]

Dengue virus enzymes are the most studied antiviral targets, including the NS2B/3 protease, NS3 NTPase and helicase activities, and the NS5 methyltransferase and RNA-dependent RNA polymerase.[116] Alternatively, ivermectin showed an inhibitory effect on the interaction of dengue virus NS5 with its nuclear transporter importin-αβ and protected against DENV 1–4 infection in vitro.[118] In 2013, a new inhibitor was identified that restricts dengue virus RNA replication by targeting of NS4B.[119] Finally, morpholino-oligonucleotides and small interfering RNAs have antiviral activity against dengue virus in vitro[120] and in vivo.[121]

Host factors

Drugs have been designed to target cellular factors such as host proteases, glucosidases, kinases, cholesterol biosynthesis pathways, and host factors involved in the immune response.[116] Celgosivir, an inhibitor of α-glucosidase, had protective efficacy in mice.[122] High-content screening of a kinase-focused library revealed anti-dengue-virus compounds that interfere with the late stage of viral infection, and drugs targeting Fyn kinase blocked virus replication.[123]

Increased levels of particular cytokines contribute to disease severity;[77] thus, reduction of dengue virus-induced cytokines might have therapeutic benefit. Treatment with tetracycline or doxycycline resulted in a substantial decline in cytokine levels in patients with dengue haemorrhagic fever/dengue shock syndrome.[124] Pentoxifylline can blunt the proinflammatory actions of TNFα, and a pilot study[125] supported potential use of pentoxifylline in severe dengue. In mice, anti-TNFα antibodies eliminate lethal disease associated with vascular leak.[126]

	Target
Viral factors	
Entry step	Domain III of DENV 2 E-glycoprotein; DC-SIGN/E-glycoprotein interaction; therapeutic monoclonal antibodies vs E; dengue virus fusion inhibitor
Viral enzymes	NS2B/3 protease; NS3 NTPase and helicase; NS5 methyltransferase; NS5 RNA-dependent RNA polymerase
Viral translation	To be determined
RNA replication	NS4B; morpholino-oligonucleotides and small interfering RNAs
Host factors	
Host enzymes	Host glucosidases; host kinases; host proteases; host cholesterol biosynthesis pathways; and host pyrimidine biosynthesis
Host factors involved in immune response or vascular leak	Cytokines (eg, TNFα; mediators of vascular leak)

DENV=dengue virus.

Table 2: **candidate antiviral drugs against dengue virus infection**

Clinical trials

Three randomised controlled trials of candidate antidengue therapeutic agents have now been completed, assessing chloroquine,[127] balapiravir,[128] and oral corticosteroid therapy.[129] Chloroquine had inhibitory effects on dengue virus replication in vitro,[54] but did not reduce the duration of viral infection, viraemia or NS1 antigenaemia in a trial, and exhibited several adverse effects.[127] Balapiravir is a prodrug of a nucleoside analogue and a polymerase inhibitor of hepatitis C virus replication in vivo. Balapiravir was well tolerated in a recent trial,[128] but it did not measurably change the kinetics of dengue virus virological markers or plasma cytokine concentrations, and did not reduce fever clearance time. The use of oral prednisolone during the early acute phase of dengue virus infection showed no effects on any of the predefined clinical, haematological, or virological endpoints in a trial, although it did not prolong viraemia or induce any adverse effects.[129] Another ongoing randomised control trial is investigating short-course lovastatin therapy in adult patients with dengue.[130] Statins (drugs developed for lipid lowering) were reported to exhibit anti-inflammatory effects at the endothelium and a possible antiviral role targeting DENV virion assembly.[131] Although these trials have not yet identified a candidate drug, they have produced an informed set of recommendations for design and conduct of early-phase clinical trials.[132]

Vector control

New approaches to vector control now exist, which are much needed, as present strategies continue to fail. An important advance is the adaptation of the endosymbiotic bacterium *Wolbachia* from *Drosophila* to *A aegypti*, which has both life-shortening effects on the mosquito and direct transmission-blocking effects on dengue virus.[133,134] The degree of invasion and fixation of *Wolbachia*-infected mosquitoes in native *A aegypti* populations and the life-shortening and dengue virus transmission-blocking activities of the *Wolbachia* strain[133] will determine the success of the intervention. Advances with genetically modified *A aegypti* carrying a dominant lethal gene (*RIDL*) and release of these male mosquitoes[135] represent another novel mosquito-targeted intervention.[136] Biological control measures are effective in reduction of *A aegypti* entomological indices.[137] New chemical products are in development to improve *A aegypti* control. Essential oils with high *A aegypti* larvicidal activity are also under investigation,[138] and some results show that silver nanoparticles synthesised by *Bacillus thuringiensis* and *Sida acuta* can be a rapid and safe biopesticide.[139] Insecticide-treated curtains and new mosquito traps have shown promise in the reduction of dengue virus infections.[140] Alternative models based on community participation in mosquito control have shown effectiveness in reduction *A aegypti* indices.[141] Advances in deciphering of genome architecture as well as phenotype-specific transcriptomics and proteomics of *A aegypti* should improve understanding of biological processes at the molecular level and serve for designing of new mosquito control strategies.[142]

Future directions

Basic and translational research in the past decade has substantially improved our knowledge about dengue; however, to contain the global pandemic, new efforts are needed. Application of nanotechnology and omics is expected to improve knowledge of virus-host and virus-vector interactions, aiding development of diagnostic techniques, therapeutic approaches, prognostic markers, new insecticides, and vaccines. Mathematical modelling should improve our understanding of transmission dynamics, vector behaviour, the impact of partially

effective vaccines, dengue burden, and cost-effectiveness of control strategies. Phylogeography of the virus and vector will provide information about movement of the virus and vector across space and time in relation to disease spread. Finally, improved understanding of natural and vaccine-induced immunity, identification of correlates of protection, interpretation of vaccine trials in terms of efficacy against both clinically apparent and inapparent dengue virus infections, and modelling of vaccine efficacy and implementation strategies are crucial for dengue containment.

In the meantime, the Global WHO Strategy for dengue prevention and control 2012–20 aims to reduce dengue mortality by at least 50% and morbidity from dengue by at least 25%.[4] The strategy promotes coordinated action among multisectoral partners, an integrated approach to vector management, and sustained control measures. Although feasible, it requires global engagement of governments, communities, and international organisations. Recognition of the severity, magnitude, and future implications of the dengue problem and strong commitment from the local to the global level, as well as support and implementation of major research findings, are required to reverse the dengue trend.

Contributors

MGG and EH contributed equally to the design, writing, and review of the Seminar.

Declaration of interests

We declare no competing interests.

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