Published Online July 8, 2010

Dengue

James Whitehorn[†] and **Jeremy Farrar**[‡*]

[†]*London School of Hygiene and Tropical Medicine, London, UK, and* [‡]*Hospital for Tropical Diseases, Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Ho Chi Minh City, Vietnam*

Introduction: Dengue is a vector-borne viral infection that endangers an estimated 2.5 billion people. Disease caused by dengue ranges from a relatively minor febrile illness to a life-threatening condition characterized by extensive capillary leak. A greater understanding of dengue has the potential to improve both the clinical management of individual cases and the control of the disease.

Sources of data: We searched the available literature using PubMed, Embase and Web of Science for relevant articles and abstracts.

Areas of agreement: Addressing our gaps in the understanding of disease pathogenesis and improving our knowledge of dengue virus biology are necessary in order to develop tools to effectively control, diagnose and treat the disease.

Areas of controversy: The pathogenesis of dengue is multifactorial and depends on both host and virus factors. A more integrated understanding of disease pathogenesis is necessary.

Areas timely for developing research: There are many questions related to disease pathogenesis, development of diagnostics, drug and vaccine development and individual case management that need addressing if the disease is to be successfully tackled.

Keywords: dengue/dengue haemorrhagic fever/dengue shock syndrome/pathogenesis/vaccine/drug development

Introduction

Accepted: June 14, 2010
**Correspondence address. Hospital for Tropical Diseases, Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Ho Chi Minh City, Vietnam. E-mail: jfarrar@oucru.org*

Dengue is a viral infection transmitted by *Aedes* mosquitoes.[1] Dengue is a *flavivirus* with four different antigenically distinct serotypes (DENV1-4).[2,3] It is a rapidly growing health problem with an estimated 2.5 billion people at risk, mainly in countries of south and south-east Asia, the Caribbean, Central and South America, and more recently in Africa.[4,5] The spread of dengue is thought to be due to a combination of factors: increased urbanization, population growth, migration and international travel and the difficulties of effective vector control.[6] Climate change may be a contributing factor in the global spread of dengue.[7]

DOI:10.1093/bmb/ldq019

It is estimated that there are between 50 and 100 million cases of dengue each year, of which ~500 000 are severe life-threatening infections.[8] There have been numerous urban outbreaks of dengue with significant health and economic impacts.[9–11] Studies in Thailand and Brazil have shown that the social and economic impact is equivalent to that of malaria in these countries.[12,13] However, globally dengue research has not received the same level of funding as other tropical infectious diseases.[8] There are currently no available drugs and no licensed vaccine, and diagnosis in endemic areas is largely clinical.[14] Dengue also poses a risk to those who travel to endemic areas and is increasingly being reported in travellers returning from trips to endemic countries.[15]

Dengue viruses cause a variety of clinical syndromes ranging from asymptomatic infection to a self-limiting febrile illness to severe dengue, a life-threatening condition characterized by increased capillary permeability and shock.[16–18] There are concerns that the old WHO definitions of dengue that are still in widespread use may lead to underdiagnosis of severe dengue.[19] In clinically apparent dengue infection symptoms develop after an incubation period of 4–7 days with an abrupt onset of fevers often accompanied by headache with severe retro-orbital pain.[20,21] Some patients develop severe arthralgia, explaining the historical name of break-bone fever.[22] Early in the illness the skin may appear flushed, with petechiae appearing during the 'critical phase' and a macular rash appearing in early convalescence. Even after uncomplicated dengue recovery may be complicated by fatigue and depression. Thrombocytopenia is almost universal in dengue infection and minor bleeding from skin and mucosal surfaces may be seen in uncomplicated infections—this can be severe in patients with peptic ulcer disease.[23] Biochemical hepatitis is frequently seen in dengue infection, but severe liver pathology, encephalitis and renal dysfunction are relatively rare.[21,24] Data from Taiwan show differences in disease manifestations between adults and children observed during an outbreak in 2002.[25] Arthralgia, headache, gastrointestinal bleeding and significantly severe dengue were more common in adult patients. The reasons for these differences are not clear but could reflect differences in the host response to infection. Significant and disabling fatigue has been described after recovery from dengue.[26] It is thought that host factors may contribute to the development of this condition. Vertical transmission of dengue can occur if infection of the mother occurs within 8 days of delivery.[27] The WHO have recently published new guidelines on dengue management.[28] The new guidelines indicate that disease caused by dengue encompasses a wide clinical spectrum and that the previously used classifications for severe dengue were not always helpful. Dengue is classified in the new system as either uncomplicated or severe.[28] Severe disease is characterized by plasma leak, haemorrhage and organ impairment. Recognizing the

warning signs for severe disease is important for successful clinical management. Warning signs include abdominal pain, evidence of fluid accumulation, hepatomegaly and increases in haematocrit accompanied by a fall in the platelet count.[28] The mechanisms that lead to severe dengue are not completely understood although various hypotheses exist. Perhaps the most widely accepted hypothesis explaining the development of severe dengue is that of antibody-mediated immune enhancement, where antibodies developed to a previous infection lead to enhanced viral uptake with a new infection of a different serotype.[29,30] This and other hypotheses will be discussed in a later section of this review.

Sources of data

We searched the available literature up to April 2010 using PubMed, Web of Science and Embase and making use of the following terms: 'dengue'; 'dengue fever'; 'dengue haemorrhagic fever (DHF)'; and 'dengue viruses'.

Areas of agreement

As dengue has emerged as a global health threat, it has received increased attention from the international public health community.[14] Essentially areas of agreement are based around addressing the lack of effective diagnostic and therapeutic options, improving clinical care of affected patients and developing tools to prevent dengue infection.

Areas of controversy

Is the virus itself or the host response to infection the main cause of severe disease?

The four serotypes of dengue can be further classified into different genotypes based on nucleotide variation.[31] Different genotypes have been associated with different levels of virulence.[32,33] It appears that the virus may evolve during epidemics to cause more severe disease as seen in disease outbreaks in Cuba and Australia.[34,35] It has been suggested that structural differences in strains of the virus lead to differing abilities to infect different cell types or cause severe disease.[36,37] However, given that only a small percentage of patients infected with dengue develop severe disease, it is clear that host factors have a major role to play in dengue pathogenesis.[31]

Many of the clinical features of dengue infection may be due to the patients' immune response. The increased vascular permeability seen in

dengue is associated with high levels of cytokines including tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin 6 (IL-6) and interleukin 2 (IL-2).[38,39] However, it is important to note that these cytokines are elevated in many diseases, yet dengue is a very specific clinical entity. Therefore the clinical features of dengue could be due to a specific constellation of these cytokines or a more complex interaction between host and virus that is yet to be characterized. Polymorphisms in TNF-α are associated with more severe disease.[40] Various other genetic factors have been associated with more severe disease: for example, polymorphisms in the vitamin D receptor gene, the mannose-binding lectin gene, and various HLA class I and II alleles.[41–44] However, none of these studies have been large enough to indicate a clear association and the question of host susceptibility remains unanswered. Cytokine dysregulation and endothelial injury contribute to the pathogenesis of yellow fever, another flavivirus.[45] The pathophysiology of severe yellow fever infection remains unclear, but given similarities with severe dengue, there may be an overlap in the host and virus determinants of susceptibility.

Is the hypothesis of antibody-mediated enhancement sufficient to explain the development of severe disease?

Epidemiological studies have shown that severe disease is more commonly seen after secondary infections.[46] Infection with one serotype is thought to result in brief protection against all serotypes. As the levels of neutralizing antibodies fall over time the non-neutralizing or sub-neutralizing antibodies form complexes with the new infecting virus and these complexes are taken up by Fc-receptor bearing cells. This results in increased uptake of virus, increased replication and viral load, and hence a postulated increased likelihood of complications.[30] This phenomenon explains the severe disease observed in infants aged between 6 and 9 months, where the low non-neutralizing levels of maternally derived antibodies are thought to play a crucial role in the development of severe disease.[47,48] These observations have led to concerns that a vaccine may potentially result in more severe disease by priming the immune system and putting individuals at risk when they are subsequently infected. These fears are ungrounded if such a vaccine produces long-term neutralizing protection to all four serotypes and those serotypes do not change over time.[3] However, severe disease can occur in primary infections and most secondary infections do not result in severe disease indicating that other factors contribute.[49–51] Direct evidence for antibody-dependent enhancement (ADE) contributing to the development of severe disease in humans has been difficult to

demonstrate, although recent work in infants and elegant *in vitro* and clinical studies have started to yield crucial insights into how ADE may contribute to pathogenesis.[51,52,53]

The pathogenesis of dengue is complex and multifactorial. No hypothesis in isolation is sufficient to explain the development of severe disease. It is the interplay between virus factors and genetically determined host factors that determine the disease outcome in the individual patient.

Growing points—areas timely for developing research

Increased understanding of dengue pathogenesis

As discussed above the mechanisms that lead to severe dengue are incompletely understood. A greater and clearer understanding of disease pathogenesis including host genetics, virus biology and immunopathology, would help the development of targeted clinical interventions.[14] Clarifying how dengue virus virulence varies between serotypes and evolves within epidemics would be helpful. Do different serotypes vary in their tissue tropism thus explaining differences in disease severity? Elucidating how the different factors within the immune system interact and contribute to disease development is essential. Too often research focuses on a single aspect of the immune response, be it antibody-mediated enhancement, T and B cell responses or the role of complement when a more integrated view, including consideration of the virus, is required.[31]

Non structural protein-1 (NS1) is a glycoprotein secreted by dengue-infected cells. Early detection of NS1 has demonstrated efficacy as a diagnostic tool.[54] Its role in dengue pathogenesis is unclear—defining this role may lend weight to its use in diagnostics. It is possible that it activates complement at endothelial surfaces thus contributing to the vascular leak that occurs in severe disease.[55] Clarifying the events that occur at the endothelial surface in dengue infection would be a major advance—the development of a suitable animal model of dengue infection would aid this progress.[56]

An increased understanding of genetic factors that contribute to disease development would help define more clearly populations at risk. Within populations an increased understanding of differing susceptibilities to disease development is necessary.[31]

Optimization of clinical management

The commonly used WHO guidelines for the management of dengue were developed after observations in children hospitalized in Bangkok

in the 1960s with dengue.[19] These guidelines have evolved over the years and have been adopted into clinical practice in endemic areas. An attempt to validate the WHO classifications for severe disease has been recently published.[57] This study showed that while the WHO classifications had good specificity for severe disease the sensitivity was lower. There is no doubt that the use of the guidelines have led to substantial mortality reductions, yet are guidelines originally developed for Thai children 40 years ago still applicable today or, for example, in the increasing number of adult patients seen globally?[19,58]

One of the difficulties diagnosing DHF using the old WHO definitions was that the criteria were too rigid.[16] Studies have shown that many cases of dengue that resulted in shock or death did not meet the WHO case definitions.[59,60] These problems have led to the development of the new WHO guidelines that categorize dengue cases into uncomplicated or severe.[28] These guidelines emphasize the importance of recognizing the warning signs of severe dengue—abdominal pain, vomiting, fluid accumulation, mucosal bleeding, lethargy or restlessness, hepatomegaly, rising haematocrit in conjunction with falling platelets.[28] These 2009 WHO guidelines include a detailed description of the clinical management of dengue including an algorithm for the management (http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf). A challenge is educating health-care workers about the new guidelines and ensuring that they result in better clinical care.

The mortality in severe dengue remains significant particularly in infants.[50,61] In addition higher mortality rates are seen when dengue newly emerges in regions where there is less experience in clinical management of cases, for example, as seen in the epidemics in India and Brazil.[10,62] Fluid management in dengue is difficult—the balance between too much and too little is critical and getting it wrong can be life-threatening.[63] Fluid resuscitation is not without risk and fluid overload in the recovery phase can cause acute respiratory distress syndrome.[28] Fluid management in patients with co-morbidities or pregnancy is difficult and would benefit from further research.

It is likely that there is a significant unreported burden of mortality in remote health facilities, where clinical management may be poor. Early and careful administration of parenteral fluids is life-saving in severe dengue. Ringer's lactate has demonstrated efficacy for use in dengue shock syndrome and dextran or starch have also been suggested for severe disease.[64] Successful management of severe dengue requires favourable staff-to-patient ratios and the ability to closely monitor patients.[28] Unfortunately the use of parenteral fluids and the intense monitoring that is required may not always be available and there is a need for research on the use of oral rehydration therapy aimed at preventing the development of shock in severe dengue.[14]

The endothelial leak seen in severe dengue is transient and if appropriate supportive therapy is given during this period, mortality rates are low.[16] However, in the early stages of disease clinical features are non-specific and are shared with other infectious aetiologies.[61] Making a diagnosis early in the disease would significantly aid clinical management. Additionally predicting which patients are at risk of severe disease would be a major advance. Traditionally diagnosis has relied on serology, which can take time to give useful results, meaning that diagnosis is often retrospective.[1] IgM against dengue can be detected after 5 days of fever—commercially available diagnostic kits based on ELISA have been assessed.[65] These have potential but have limitations, for example, the persistence of IgM in some patients in endemic areas making it difficult to diagnose an acute infection. The detection of the dengue protein NS1 also has proven use in diagnosis.[54] Quantifying the titre of NS1 early in clinical illness may allow one to predict those at risk of developing severe disease.[66] Real-time PCR is another potential, highly sensitive diagnostic tool in dengue diagnosis.[67] However, the cost of molecular tests such as PCR make it an impractical tool for use in many settings where dengue is prevalent. A rapid sensitive test that combines detection of NS1, IgG and IgM allowing diagnosis of infection throughout the illness course would be a major advance in dengue diagnostics. The development of appropriate clinical algorithms for use in resource-limited settings in parallel to the development of molecular diagnostic tools is necessary if the burden of dengue is to be adequately dealt with. A study has demonstrated the potential use of a decision tree algorithm using simple clinical and haematological parameters.[68] Testing the validity of such an algorithm in primary care settings have the potential to improve the triage and initial clinical management of patients with dengue. Detection of plasma leakage through serial ultrasound is a useful adjunctive tool in dengue diagnosis and management, and yet it is unlikely to be of benefit in many areas due to lack of availability.[69] Identifying additional predictors of dengue would be useful, but making these into diagnostic techniques that are practical in resource-limited settings is the challenge.[70]

Vaccine development

A vaccine against dengue would be a major advance in the control of the disease. In view of the potential risks of antibody-mediated enhancement seen with heterotypic infections, a successful vaccine would have to offer lasting protection against all four serotypes.[2] Based on the success of vaccination against other flaviviruses, for example, Japanese encephalitis and yellow fever, the development of a

live attenuated vaccine had previously been the most promising prospect for vaccinologists.[71] However despite encouraging results with monovalent vaccines in clinical trials the development of a tetravalent formulation has proved problematic and development has been suspended.[3,72–75] The leading vaccine candidate at present is a chimeric vaccine—a recombinant clone based on yellow fever vaccine strain with dengue virus membrane and envelope protein genes substituted into the construct.[76,77] This chimeric vaccine has shown promise in Phase II clinical trials and appears safe and immunogenic. It is anticipated that Phase III trials will start in the near future.

Despite promising progress there remain unanswered questions that need to be addressed and are potential areas for research. The lack of a suitable animal model of dengue infection hinders the transfer of laboratory findings into clinical practice.[31] How do we quantify immune protection? Do tetravalent vaccine constructs hinder the development of immunity to individual serotypes? Could a dengue vaccine render populations more vulnerable to severe infection, either through antibody-mediated enhancement or via a mechanism similar to that seen with vaccines against respiratory syncytial virus?[78] How safe are dengue vaccines in different subsets of the population?[3]

In addition to the science of vaccine development and questions regarding the safety and efficacy of individual vaccine candidates, there needs to be sustained co-ordination between different countries and vaccine developers if a vaccine is to be successfully developed.[53,79]

Anti-dengue drugs

A greater understanding of dengue virus biology has meant that targets within the lifecycle have been identified that could potentially be the site of a therapeutic agent.[80] The staggering success in developing drugs against HIV is an example of how efficiently effective antivirals can be developed given appropriate funding.

One potential mechanism of action of an anti-dengue drug is through inhibition of viral entry. The fusion of the viral membrane with the host membrane is mediated by dengue virus E protein.[81] Research has shown some promising initial results in laboratory studies with an experimental entry inhibitor—these findings could pave the way to the development of successful therapeutic agents.[82] Other potential targets receiving research attention are the viral proteins NS3 and NS5, which play an integral role in genome replication—their protease domains could be target for protease inhibitors.[83,84] A computer program has been used to identify suitable molecules that can 'dock' into the NS3 protease domain.[85] The challenge is to bridge the gap between findings in the laboratory and the

bedside; however, given the progress in our understanding of dengue virus biology, the future of drug development is encouraging. There are potential therapeutic developments for the treatment of other flaviviruses such as hepatitis C.[86] In view of structural similarities between different flaviviruses, these developments could be used in the field of dengue treatment. The complications of dengue occur as the virus is cleared from the blood, and so it is possible that an anti-dengue drug will have no beneficial role. As the severe manifestations of disease are in part due to the immune response perhaps the development of an immunomodulatory agent would be the best therapeutic strategy? Animal studies have shown the protective effect of monoclonal antibodies against the NS1 protein of West Nile virus, and more recent work in mice has shown the therapeutic potential of a monoclonal antibody directed against a structural epitope in an experimental DENV-1 infection.[87,88]

Vector control

In addition to vaccination, successful vector control would be a useful adjunct to controlling dengue. Dengue is closely associated with *Aedes aegypti*—the global spread of dengue is related to changes in human behaviour, in particular the expansion of large urban centres, which support the breeding of *A. aegypti*.[6] Although vector control has previously had success, the persistence of pockets of mosquitoes resulted in disease re-emergence.[89] Studies have shown different efficiencies of dengue transmission between different mosquito strains and different serotypes of dengue.[90,91] A greater understanding of the interactions between mosquitoes and the different serotypes of dengue would give us a greater understanding of transmission dynamics and would potentially lead to better prediction of epidemics, and thus better control of the disease. The development of genetically modified mosquitoes that are less able to transmit dengue is a potential option for disease control. However, mathematical models have indicated that this strategy may select for greater disease virulence, suggesting that measures that increase mosquito mortality may be more effective.[92] Transgenic mosquitoes are likely to be less reproductively fit—infection of transgenic mosquitoes with the symbiont Wolbachia potentially confers a reproductive advantage, thus allowing the establishment of transgenics in the environment.[93] Cyclopoid copepods are invertebrate predators of mosquito larvae. Introduction of copepods into water containers, the typical breeding site of *Aedes*, can have a major impact on mosquito populations.[94] The roll-out of larvicidal interventions requires active community involvement and the success of such measures would depend on imaginative public health engagement.[94]

Conclusions

Successfully tackling the threat of dengue represents a major public health challenge for the twenty-first century. A co-ordinated multidisciplinary approach is necessary. Major advances are possible provided dengue research receives the attention and funding its prevalence deserves.

References

1 Halstead SB. Dengue. *Lancet* 2007;**370**:1644–52.
2 Halstead SB. Pathogenesis of dengue: challenges to molecular biology. *Science* 1988;**239**:476–81.
3 Webster DP, Farrar J, Rowland-Jones S. Progress towards a dengue vaccine. *Lancet Infect Dis* 2009;**9**:678–87.
4 DengueNet–WHO's Internet-based system for the global surveillance of dengue fever and dengue haemorrhagic fever (dengue/DHF). Dengue/DHF—global public health burden. *Wkly Epidemiol Rec* 2002;**77**:300–4. http://www.who.int/denguenet.
5 Franco L, Caro AD, Carletti F *et al*. Recent expansion of dengue virus serotype 3 in West Africa. *Euro Surveill* 2010;**15**:19490.
6 Gubler DJ. The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle? *Comp Immunol Microbiol Infect Dis* 2004;**27**:319–30.
7 Hsieh YH, Chen CW. Turning points, reproduction number, and impact of climatological events for multi-wave dengue outbreaks. *Trop Med Int Health* 2009;**14**:628–38.
8 Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol* 2002;**10**:100–3.
9 Gubler DJ. Cities spawn epidemic dengue viruses. *Nat Med* 2004;**10**:129–30.
10 Anuradha S, Singh NP, Rizvi SN *et al*. The 1996 outbreak of dengue hemorrhagic fever in Delhi, India. *Southeast Asian J Trop Med Public Health* 1998;**29**:503–6.
11 Vaughn DW. Invited commentary: dengue lessons from Cuba. *Am J Epidemiol* 2000;**152**:800–3.
12 Clark DV, Mammen MP Jr, Nisalak A *et al*. Economic impact of dengue fever/dengue hemorrhagic fever in Thailand at the family and population levels. *Am J Trop Med Hyg* 2005;**72**:786–91.
13 Luz PM, Grinsztejn B, Galvani AP. Disability adjusted life years lost to dengue in Brazil. *Trop Med Int Health* 2009;**14**:237–46.
14 Farrar J, Focks D, Gubler D *et al*. Towards a global dengue research agenda. *Trop Med Int Health* 2007;**12**:695–9.
15 Allwinn R, Hofknecht N, Doerr HW. Dengue in travellers is still underestimated. *Intervirology* 2008;**51**:96–100.
16 WHO. *Dengue haemorrhagic fever: diagnosis, treatment, prevention and control*. 2nd Edition Geneva: World Health Organisation, 1997, ed.
17 WHO. In Asia ROfS-E (eds) *Guidelines for treatment of dengue fever/dengue haemorrhagic fever in small hospitals*. New Delhi: World Health Organisation, 1999, editor.
18 Kalayanarooj S, Vaughn DW, Nimmannitya S *et al*. Early clinical and laboratory indicators of acute dengue illness. *J Infect Dis* 1997;**176**:313–21.
19 Deen JL, Harris E, Wills B *et al*. The WHO dengue classification and case definitions: time for a reassessment. *Lancet* 2006;**368**:170–3.
20 Endy TP, Chunsuttiwat S, Nisalak A *et al*. Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng Phet, Thailand. *Am J Epidemiol* 2002;**156**:40–51.

21 Kabra SK, Jain Y, Singhal T *et al*. Dengue hemorrhagic fever: clinical manifestations and management. *Indian J Pediatr* 1999;**66**:93–101.
22 McBride JH. Dengue fever. An Australian perspective. *Aust Fam Physician* 1999;**28**:319–23.
23 tsai CJ, Kuo CH, Chen PC *et al*. Upper gastrointestinal bleeding in dengue fever. *Am J Gastroenterol* 1991;**86**:33–5.
24 thisyakorn U, Thisyakorn C. Dengue infection with unusual manifestations. *J Med Assoc Thai* 1994;**77**:410–3.
25 Wang C, Lee I, Su M *et al*. Differences in clinical and laboratory characteristics and disease severity between children and adults with dengue virus infection in Taiwan, 2002. *Trans R Soc Trop Med Hyg* 2009;**103**:871–7.
26 Seet RC, Quek AM, Lim EC. Post-infectious fatigue syndrome in dengue infection. *J Clin Virol* 2007;**38**:1–6.
27 Chye JK, Lim CT, Ng KB *et al*. Vertical transmission of dengue. *Clin Infect Dis* 1997;**25**:1374–7.
28 WHO. *Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control*, new edn. Geneva: World Health Organisation, 2009.
29 Kliks SC, Nisalak A, Brandt WE *et al*. Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. *Am J Trop Med Hyg* 1989;**40**:444–51.
30 Halstead SB. Neutralization and antibody-dependent enhancement of dengue viruses. *Adv Virus Res* 2003;**60**:421–67.
31 Martina BE, Koraka P, Osterhaus AD. Dengue virus pathogenesis: an integrated view. *Clin Microbiol Rev* 2009;**22**:564–81.
32 Leitmeyer K, Vaughn D, Watts D *et al*. Dengue virus structural differences that correlate with pathogenesis. *J Virol* 1999;**73**:4738–47.
33 Sanchez I, Ruiz B. A single nucleotide change in the E protein gene of dengue virus 2 Mexican strain affects neurovirulence in mice. *J Gen Virol* 1996;**77**:2541–5.
34 Streatfield R, Bielby G, Sinclair D. A primary dengue 2 epidemic with spontaneous haemorrhagic manifestations. *Lancet* 1993;**342**:560–1.
35 Guzman MG, Kouri G, Halstead SB. Do escape mutants explain rapid increases in dengue case-fatality rates within epidemics? *Lancet* 2000;**355**:1902–3.
36 Vaughn DW, Green S, Kalayanarooj S *et al*. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000;**181**:2–9.
37 Diamond M, Edgil D, Roberts T *et al*. Infection of human cells by dengue virus is modulated by different cell types and viral strains. *J Virol* 2000;**74**:7814–23.
38 Kurane I, Innis BL, Nimmannitya S *et al*. Activation of T lymphocytes in dengue virus infections. High levels of soluble interleukin 2 receptor, soluble CD4, soluble CD8, interleukin 2, and interferon-gamma in sera of children with dengue. *J Clin Invest* 1991;**88**:1473–80.
39 Hober D, Poli L, Roblin B *et al*. Serum levels of tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 beta) in dengue-infected patients. *Am J Trop Med Hyg* 1993;**48**:324–31.
40 Fernandez-Mestre MT, Gendzekhadze K, Rivas-Vetencourt P *et al*. TNF-alpha-308A allele, a possible severity risk factor of hemorrhagic manifestation in dengue fever patients. *Tissue Antigens* 2004;**64**:469–72.
41 Acioli-Santos B, Segat L, Dhalia R *et al*. MBL2 gene polymorphisms protect against development of thrombocytopenia associated with severe dengue phenotype. *Hum Immunol* 2008;**69**:122–8.
42 LaFleur C, Granados J, Vargas-Alarcon G *et al*. HLA-DR antigen frequencies in Mexican patients with dengue virus infection: HLA-DR4 as a possible genetic resistance factor for dengue hemorrhagic fever. *Hum Immunol* 2002;**63**:1039–44.
43 Loke H, Bethell D, Phuong CX *et al*. Susceptibility to dengue hemorrhagic fever in Vietnam: evidence of an association with variation in the vitamin d receptor and Fc gamma receptor IIa genes. *Am J Trop Med Hyg* 2002;**67**:102–6.
44 Loke H, Bethell DB, Phuong CX *et al*. Strong HLA class I–restricted T cell responses in dengue hemorrhagic fever: a double-edged sword? *J Infect Dis* 2001;**184**:1369–73.
45 Monath T, Barrett A. Pathogenesis and pathophysiology of yellow fever. *Adv Virus Res* 2003;**60**:343–95.

46 Graham RR, Juffrie M, Tan R *et al*. A prospective seroepidemiologic study on dengue in children four to nine years of age in Yogyakarta, Indonesia I. Studies in 1995–1996. *Am J Trop Med Hyg* 1999;**61**:412–9.
47 Kalayanarooj S, Nimmannitya S. Clinical presentations of dengue hemorrhagic fever in infants compared to children. *J Med Assoc Thai* 2003;**86**:S673–80.
48 Chau TN, Quyen NT, Thuy TT *et al*. Dengue in Vietnamese infants—results of infection-enhancement assays correlate with age-related disease epidemiology, and cellular immune responses correlate with disease severity. *J Infect Dis* 2008;**198**:516–24.
49 Guzman MG, Kouri G. Dengue: an update. *Lancet Infect Dis* 2002;**2**:33–42.
50 Wichmann O, Hongsiriwon S, Bowonwatanuwong C *et al*. Risk factors and clinical features associated with severe dengue infection in adults and children during the 2001 epidemic in Chonburi, Thailand. *Trop Med Int Health* 2004;**9**:1022–9.
51 Dejnirattisai W, Jumnainsong A, Onsirisakul N *et al*. Cross-reacting antibodies enhance dengue virus infection in humans. *Science* 2010;**328**:745–8.
52 Chau T, Anders K, Lien lB *et al*. Clinical and virological features of dengue in Vietnamese infants. *PLoS Negl Trop Dis* 2010;**4**:e657.
53 Stephenson JR. Understanding dengue pathogenesis: implications for vaccine design. *Bull World Health Organ* 2005;**83**:308–14.
54 Hang VT, Nguyet NM, Trung DT *et al*. Diagnostic accuracy of NS1 ELISA and lateral flow rapid tests for dengue sensitivity, specificity and relationship to viraemia and antibody responses. *PLoS Negl Trop Dis* 2009;**3**:e360.
55 Avirutnan P, Punyadee N, Noisakran S *et al*. Vascular leakage in severe dengue virus infections: a potential role for the nonstructural viral protein NS1 and complement. *J Infect Dis* 2006;**193**:1078–88.
56 Rico-Hesse R. Dengue virus markers of virulence and pathogenicity. *Future Virol* 2009;**4**:581.
57 Srikiathachorn A, Gibbons R, Green S *et al*. Dengue haemorrhagic fever: the sensitivity and specificity of the World Health Organisation definitions for identification of severe cases of dengue in Thailand, 1994–2005. *Clin Infect Dis* 2010;**50**:1135–43.
58 Kalayanarooj S. Standardised clinical management: evidence of reduction of dengue haemorrhagic fever case-fatality rate in Thailand. *Dengue Bull* 1999;**23**:10–7.
59 Sumarmo, Wulur H, Jahja E *et al*. Clinical observations on virologically confirmed fatal dengue infections in Jakarta, Indonesia. *Bull World Health Organ* 1983;**61**:693–701.
60 Phuong CX, Nhan NT, Kneen R *et al*. Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: is the world health organization classification system helpful? *Am J Trop Med Hyg* 2004;**70**:172–9.
61 Harris E, Videa E, Perez L *et al*. Clinical, epidemiologic, and virologic features of dengue in the 1998 epidemic in Nicaragua. *Am J Trop Med Hyg* 2000;**63**:5–11.
62 Teixeira MG, Costa Mda C, Barreto F *et al*. Dengue: twenty-five years since reemergence in Brazil. *Cad Saude Publica* 2009;**25**:S7–18.
63 Moxon C, Wills B. Management of severe dengue in children. *Adv Exp Med Biol* 2008;**609**:131–44.
64 Wills BA, Nguyen MD, Ha TL *et al*. Comparison of three fluid solutions for resuscitation in dengue shock syndrome. *N Engl J Med* 2005;**353**:877–89.
65 Hunsperger EA, Yoksan S, Buchy P *et al*. Evaluation of commercially available anti-dengue virus immunoglobulin M tests. *Emerg Infect Dis* 2009;**15**:436–40.
66 Libraty DH, Young PR, Pickering D *et al*. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *J Infect Dis* 2002;**186**:1165–8.
67 Kong YY, Thay CH, Tin TC *et al*. Rapid detection, serotyping and quantitation of dengue viruses by TaqMan real-time one-step RT-PCR. *J Virol Methods* 2006;**138**:123–30.
68 Tanner L, Schreiber M, Low JG *et al*. Decision tree algorithms predict the diagnosis and outcome of dengue fever in the early phase of illness. *PLoS Negl Trop Dis* 2008;**2**:e196.
69 Srikiatkhachorn A, Krautrachue A, Ratanaprakarn W *et al*. Natural history of plasma leakage in dengue hemorrhagic fever: a serial ultrasonographic study. *Pediatr Infect Dis J* 2007;**26**:283–90; discussion 91–2.
70 Srikiatkhachorn A, Green S. Markers of dengue disease severity. *Curr Top Microbiol Immunol* 2010;**338**:67–82.

71 Edelman R. Dengue vaccines approach the finish line. *Clin Infect Dis* 2007;**45**:S56–60.
72 Innis BL, Eckels KH. Progress in development of a live-attenuated, tetravalent dengue virus vaccine by the United States Army Medical Research and Materiel Command. *Am J Trop Med Hyg* 2003;**69**:1–4.
73 Edelman R, Wasserman SS, Bodison SA *et al*. Phase I trial of 16 formulations of a tetravalent live-attenuated dengue vaccine. *Am J Trop Med Hyg* 2003;**69**:48–60.
74 Sun W, Edelman R, Kanesa-Thasan N *et al*. Vaccination of human volunteers with monovalent and tetravalent live-attenuated dengue vaccine candidates. *Am J Trop Med Hyg* 2003;**69**:24–31.
75 Halstead SB, Marchette NJ. Biologic properties of dengue viruses following serial passage in primary dog kidney cells: studies at the University of Hawaii. *Am J Trop Med Hyg* 2003;**69**:5–11.
76 Guirakhoo F, Kitchener S, Morrison D *et al*. Live attenuated chimeric yellow fever dengue type 2 (ChimeriVax-DEN2) vaccine: Phase I clinical trial for safety and immunogenicity: effect of yellow fever pre-immunity in induction of cross neutralizing antibody responses to all 4 dengue serotypes. *Hum Vaccin* 2006;**2**:60–7.
77 Guirakhoo F, Pugachev K, Zhang Z *et al*. Safety and efficacy of chimeric yellow Fever-dengue virus tetravalent vaccine formulations in nonhuman primates. *J Virol* 2004;**78**:4761–75.
78 Castilow E, Olson M, Varga S. Understanding respiratory syncytial virus (RSV) vaccine-enhanced disease. *Immunol Res* 2007;**39**:225–39.
79 Edelman R, Hombach J. "Guidelines for the clinical evaluation of dengue vaccines in endemic areas": summary of a World Health Organisation Technical Consultation. *Vaccine* 2008;**26**:4113–9.
80 Swaminathan S, Khanna N. Dengue: recent advances in biology and current status of translational research. *Curr Mol Med* 2009;**9**:152–73.
81 Heinz F, Allison S. The machinery for flavivirus fusion with host cell membranes. *Curr Opin Microbiol* 2001;**4**:450–5.
82 Wang Q, Patel S, Vangrevelinghe E *et al*. A small-molecule dengue virus inhibitor. *Antimicrob Agents Chemother* 2009;**53**:1823–31.
83 Lescar J, Luo D, Xu T *et al*. Towards the design of antiviral inhibitors against flaviviruses: the case for the multifunctional NS3 protein from Dengue virus as a target. *Antiviral Res* 2008;**80**:94–101.
84 Melino S, Paci M. Progress for dengue virus diseases. Towards the NS2B-NS3pro inhibition for a therapeutic-based approach. *FEBS J* 2007;**274**:2986–3002.
85 Tomlinson SM, Malmstrom RD, Watowich SJ. New approaches to structure-based discovery of dengue protease inhibitors. *Infect Disord Drug Targets* 2009;**9**:327–43.
86 Webster D, Klenerman P, Collier J *et al*. Development of novel treatments for hepatitis C. *Lancet Infect Dis* 2009;**9**:108–17.
87 Chung K, Nybakken G, Thompson B *et al*. Antibodies against West Nile Virus nonstructural protein NS1 prevent lethal infection through Fc gamma receptor-dependent and -independent mechanisms. *J Virol* 2006;**80**:1340–51.
88 Shrestha B, Brien J, Sukupolvi-Petty S *et al*. The development of therapeutic antibodies that neutralize homologous and heterologous genotypes of dengue virus type 1. *PLoS Pathog* 2010;**6**:e1000823.
89 Ooi EE, Goh KT, Gubler DJ. Dengue prevention and 35 years of vector control in Singapore. *Emerg Infect Dis* 2006;**12**:887–93.
90 Gubler DJ, Nalim S, Tan R *et al*. Variation in susceptibility to oral infection with dengue viruses among geographic strains of Aedes aegypti. *Am J Trop Med Hyg* 1979;**28**:1045–52.
91 Hanley K, Nelson J, Schirtzinger E *et al*. Superior infectivity for mosquito vectors contributes to competitive displacement among strains of dengue virus. *BMC Ecol* 2008;**8**:1.
92 Medlock J, Luz PM, Struchiner CJ *et al*. The impact of transgenic mosquitoes on dengue virulence to humans and mosquitoes. *Am Nat* 2009;**174**:565–77.
93 Rasgon J. Using predictive models to optimize Wolbachia-based strategies for vector-borne disease control. *Adv Exp Med Biol* 2008;**627**:114–25.
94 Marten G, Reid J. Cyclopoid copepods. *J Am Mosq Control Assoc* 2007;**23**:65–92.