Dengue haemorrhagic fever: questions of pathogenesis

Mary Jane Cardosa

The year under review has seen a remarkable proliferation of papers on dengue. Four prospective studies have been carried out across the dengue belt, many groups have been pushing at the question of pathogenesis of dengue haemorrhagic fever, and a breakthrough has been achieved in the development of a mouse model for human dengue haemorrhagic fever. Curr Opin Infect Dis 13:471–475.

Institute of Health and Community Medicine, Universiti Malaysia Sarawak, Sarawak, Malaysia

Correspondence to Mary Jane Cardosa, Institute of Health and Community Medicine, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
Tel: +60 82 671 730; fax: +60 82 671 903; e-mail: jcardosa@mailhost.unimas.my

Current Opinion in Infectious Diseases 2000, 13:471–475

Abbreviations

ADE	antibody-dependent enhancement
CNS	central nervous system
CSF	cerebrospinal fluid
DHF	dengue haemorrhagic fever
DSS	dengue shock syndrome
JEV	Japanese encephalitis virus
RT–PCR	reverse transcriptase–polymerase chain reaction
WHO	World Health Organization

0951-7375

Introduction

The four dengue virus serotypes (DEN-1 to DEN-4) have co-circulated in Southeast Asia for several decades, with the first recognised epidemic of dengue haemorrhagic fever (DHF) in Manila, the Philippines, in 1953/1954. In the next two decades, epidemic DHF spread throughout Southeast Asia to become a leading cause of morbidity and mortality among children throughout the region. In the latter years of the 1970s to the 1980s, epidemiological and clinical research on DHF was centred in Bangkok, Thailand, where the insightful studies of local doctors (most notably Dr Suchitra Nimannitya of Children's Hospital, Bangkok), in collaboration with other scientists established the basis for the World Health Organization (WHO) classification for grading DHF, first published in 1986 [1] and later updated in 1997 [2].

DHF is thus distinguished from classical dengue fever by the presence of the hallmarks of haemorrhagic fever, characterized by vascular permeability and consequently haemoconcentration. It is important to recognize that DHF is not merely a febrile disease with haemorrhagic manifestations, however severe, but is a qualitatively distinct, potentially life-threatening leaky capillary syndrome. The WHO criteria for making a diagnosis of DHF are clear: it is a febrile illness with thrombocytopenia of $100\,000 \times 10^6$/l or less and a haematocrit raised 20% or more above the norm. In patients with DHF grade I, a positive tourniquet test is the only haemorrhagic manifestation, whereas in DHF grade II, spontaneous bleeding occurs. Grades III and IV are also referred to as dengue shock syndrome (DSS). When patients experience circulatory failure with narrowing of the pulse pressure, and a rapid and weak pulse, they are said to have DHF grade III, whereas those in profound shock with no detectable blood pressure and pulse have DHF grade IV [1,2].

Neurological dengue

Besides the well characterised dengue fever and DHF, it has long been recognised that patients with dengue infection may present with 'unusual manifestations', including fulminant hepatitis, encephalopathy and encephalitis [3–12]. Although most of these reports are case descriptions rather than prospective clinical studies, Solomon and coworkers [13••] have now corrected this deficiency in an excellent study of the neurological manifestations of dengue in Vietnam. Interestingly, this was not designed as a study of dengue infection

specifically, but rather a study of central nervous system (CNS) infections in children and adults. Sixteen out of 378 (4.2%) patients with CNS infections during the study period (1995) had evidence of dengue virus infection compared with four out of 286 (1.4%) hospital control subjects who had been admitted with diphtheria (children) or typhoid (adults). In addition to the 16 patients identified in the 1995 study period, Solomon and coworkers [13••] also included another five patients identified subsequently, and the paper goes on to discuss these 21 patients. Twelve of these patients did not have any characteristic features of dengue on admission; 10 were encephalopathic and the authors ask the question: 'Should such patients be considered to have dengue encephalitis?' However, seven of the nine patients with a neurological diagnosis of 'encephalitis' (as opposed to encephalopathy or hepatic encephalopathy) had no classical features of dengue infection.

In a dengue endemic area, during a dengue season, a high rate of inapparent or subclinical dengue infection occurs, and it is therefore necessary to rule out the possibility that the dengue infection detected in such cases is merely coincidental. The authors have attempted to do this fairly convincingly for a range of possible aetiologies. Most important of these is Japanese encephalitis virus (JEV), which is highly endemic in Vietnam and other parts of Southeast Asia, and is probably the most important cause of encephalitis in the region [14]. No JEV was grown from the serum or cerebrospinal fluid (CSF) of these patients, but it is extremely rare to grow JEV from peripheral specimens, even from patients with confirmed Japanese encephalitis. Indeed, JEV isolates from human infection are best obtained from brain tissue at autopsy [15]. However, it is possible to demonstrate the absence of JEV genome in the CSF by reverse transcriptase–polymerase chain reaction (RT–PCR), and it is a pity the study did not include this very important evidence to make a more convincing argument that the seven encephalitis cases with no features of dengue fever or DHF probably did indeed have dengue encephalitis.

The issue, however, is not whether dengue infection can cause neurological disease, but whether dengue viruses can replicate in the CNS in a natural infection. None of the putative 'dengue encephalitis' cases in the Vietnam study had a CSF isolate, although two of these did have evidence of dengue genome in the CSF by RT–PCR. Rosen and coworkers [16••] provided an answer to this very question. Tissues from 18 fatal DHF cases were tested for the presence of dengue virus genome by RT–PCR, and the findings (14 out of 16 liver specimens had dengue virus RNA) confirmed earlier studies suggesting dengue virus infection of the liver [17,18]. However, the absence of dengue virus RNA in all 44 samples of brain tissue from 15 patients is noteworthy. The authors point out that encephalopathy is common in severe dengue and that 12 of the patients in the study did have convulsions or encephalitis, thus confirming that dengue virus replication in the CNS, if it does occur, must be rare.

Dengue haemorrhagic fever criteria and disease severity

In this context, it is important to realise that the WHO criteria for the diagnosis of dengue fever and DHF were meant to underscore the new clinical entity (DHF) recognised in Southeast Asia at that time, and to provide the guidelines for standardised grading, not of disease severity as such, but of the spectrum of the manifestation of haemorrhagic fever. That patients may present with hepatic or neurological or other manifestations does not negate these very well established and accepted criteria. Therefore recent attempts to introduce different case definitions [19•,20] should be approached with caution. Murgue and coworkers [19•] were concerned that the WHO classification did not adequately account for disease severity, and provided data to show that in a retrospective study of laboratory confirmed dengue infections, six out of 10 fatal cases were classified as dengue fever, and fatality in these dengue fever cases was attributed to multisystem failure, particularly severe hepatic disorders. Rigau-Perez and Bonilla [20], on the other hand, were concerned with obtaining more sensitive definitions for DHF, but were well aware that any modified case definitions should be evaluated in prospective studies before general adoption.

Nearly three decades after the first Southeast Asian DHF epidemic, South America experienced its first DHF epidemic in Cuba in 1981. This was followed in 1989 by Venezuela, and then Brazil in 1990. DHF is now endemic in many countries in the Americas [21]. Most of the DHF cases seen in this emerging problem in the Americas have been associated with DEN-2. Rico-Hesse and coworkers [22] showed that there has been an introduction of Southeast Asian genotypes to the Americas, and postulated that this may account for the increase in DHF in these countries in the past decade. The conclusions of their study have been strengthened by more recent data showing that there are sequence differences in the 5′NTR and the 3′NTR of Southeast Asian and American genotypes of DEN-2 that give rise to different secondary structures at these two ends of the viral genome [23•]. Although it is intriguing that isolates from two DHF cases (Jamaica in 1983 and Venezuela in 1990) belonged to the Southeast Asian genotype, whereas isolates from dengue fever cases (Venezuela 1987, Mexico 1992, Peru 1995, Peru 1996) all belonged to the American genotype, many more strains need to be analysed before any firm conclusions can be made.

The spread of DHF to the Caribbean and to the Americas has paved the way for several prospective epidemiological studies in the spirit of the Rayong study in Thailand in the early 1980s [24]. Watts and coworkers [25••] studied dengue infection in Iquitos, Peru, from 1990. An epidemic of mild dengue (predominantly DEN-2) was recognized between May and October 1995. As there had been serological evidence of DEN-1 infection previously, this outbreak provided an opportunity to investigate sequential DEN-1 to DEN-2 infection. Unlike the Rayong study, in which a fifth of the children with sequential DEN-1 to DEN-2 infection had DSS, in Iquitos no DHF was observed. The authors ascribed this finding to the fact that the DEN-2 viruses isolated in the Peru outbreak of 1995 were of the American genotype, and probably did not have the potential to cause DHF.

Do we finally have a handle on pathogenesis then? Is it going to be as simple as this – that Southeast Asian genotypes have DHF-causing potential and others do not? Preliminary data from the first year of another prospective study [26•], in Yogyakarta, Indonesia, revealed that the DEN-1 to DEN-2 sequential infection experienced by 31 children in this Southeast Asian town did not yield any DHF. Clearly more work needs to be done. The DEN-2 viruses from the study need to be sequenced and genotyped, for it is conceivable that the American genotype is also found in Southeast Asia. Alternatively, the master key to understanding the pathogenesis of DHF is not yet in our hands.

Viraemia and disease severity

In a fourth prospective study published during this review period, Murgue and coworkers [27] investigated the duration and magnitude of viraemia in children in a DEN-2 outbreak in French Polynesia in 1996–1997. Using the presence of viral RNA in the plasma as a surrogate for viraemia, the investigators determined that: (i) the mean duration of viraemia was lower in dengue fever than in DHF patients; (ii) the mean viraemia titres were higher in patients with DHF than those with dengue fever; (iii) both the above mean values were not significantly different between primary and secondary dengue. In seven patients, virus RNA was still detectable in the last sample (one on day 4 and six on day 5). These patients seem to have been excluded from the analysis, and if so, the mean duration of viraemia (overall mean 4.4 ± 0.9 days), as determined by RT–PCR, could actually be longer than estimated. Depending on how these seven cases were distributed between the dengue fever and DHF categories, the determinations of mean duration and the magnitude of viraemia could tell rather different stories. The authors concluded that their results did not support the infection enhancement hypothesis. I fail to understand why.

Vaughn and coworkers [28••] investigated viraemia titres in serial plasma samples from 168 children in Bangkok who had dengue virus infection. This work involved tremendous effort because the quantitation and duration of viraemia was determined by inoculating dilutions of plasma samples intrathoracically into mosquitoes. Unlike PCR-based methods that determine the presence of viral RNA, whether associated with viable virus or not, the methods used in that study actually gave a picture of how much viable virus was present. The results showed that viraemia during primary infection was prolonged compared with secondary infections. This is in contrast to the data of Murgue and coworkers [27], but can easily be explained if we consider the fact that in a secondary immune response to dengue infection there will be circulating antibody binding to and inactivating virus at later stages in the course of the illness. These inactivated viruses would be detected in a PCR-based assay but not in a biological assay. The study also showed that the rate of virus clearance was faster in patients experiencing secondary compared with primary infection, and was also faster in those with DHF than those with dengue fever. Incidentally, data from the study also confirm that at the time of defervescence, when the platelet counts drop and the characteristic vascular permeability occurs in DHF, there is little or no viable virus in the circulation, giving further credibility to the argument that these events are mediated by processes not directly related to infection, but rather to mediators such as cytokines [29,30•].

Another finding from the Bangkok study [28••] is that the higher the peak viraemia titre, the greater the pleural effusion. If pleural effusion is taken as a measure of the severity of DHF, these data show that higher viraemia does indeed occur in children with severe DHF. Both peak viraemia titre and secondary immune responses were shown to correlate with the severity of DHF. As the authors pointed out, this is consistent with both of the dominant hypotheses of dengue pathogenicity: a virulent virus might be expected to replicate to higher titres, just as antibody-dependent enhancement (ADE) is expected to produce higher viraemias in patients with secondary immune responses.

We are fortunate that the fascinating problem of how co-circulating dengue virus serotypes may cause such a wide spectrum of disease has caught the attention of eminent epidemiologist Anderson. In two papers from his group [31,32], the effect of ADE on transmission dynamics was explored using mathematical models. The take-home message is that ADE may explain those cyclical dengue epidemics we are so familiar with in Southeast Asia [31]. Furthermore, statistically rigorous methods applied to data from Thailand supported a role for ADE in the observed epidemiological patterns [32].

Despite the advances made in recent years, the pathogenesis of DHF remains poorly understood. It would seem that even if some dengue viruses have DHF-causing potential and others might not, in the quest for a vaccine against dengue, we would do well to account for the immune potentiation effects of sequential dengue infection. It is thus imperative that suitable animal models are developed for testing vaccine candidates, and that this testing involves not merely the measurement of protective responses in a relatively short period of time, as described by Velzing and coworkers [33•] in their comparison of protective immunity in a monkey model. In that study, involving three groups of two cynomolgus monkeys, each pair was either immunized with a candidate live attenuated vaccine, a recombinant baculovirus subunit vaccine consisting of the envelope protein of DEN-2, or was mock-immunised with phosphate buffered saline. The humoral immune response of each of the monkeys was carefully tracked by enzyme-linked immunosorbent assay, haemagglutination inhibition tests and virus neutralization tests before and after challenge. The presence of virus after challenge was determined using RT–PCR as well as virus isolation, and the authors concluded that the live attenuated candidate vaccine did indeed protect the monkeys against virus challenge, whereas the envelope protein subunit vaccine may not have been potent enough to protect. It was most interesting that in one of the two monkeys receiving the subunit vaccine, virus was isolated 9 days after challenge, whereas both control monkeys no longer had virus detected either by RT–PCR or by isolation by this time. Immune potentiation?

Mouse model

An elegant mouse model for dengue virus infection has now been described [34••], which may be useful in studying various aspects of the pathogenesis of DHF. A human hepatocarcinoma cell line, HepG2, known to support dengue virus replication [35,36] was transplanted into severe combined immunodeficient (SCID) mice. Such HepG2-grafted mice were infected with DEN-2 virus, and the authors showed very convincingly that the mice developed many of the features of DHF, including thrombocytopenia and increased haematocrit. Virus inoculated intraperitoneally was detected in the serum and in the liver, and also induced paralysis, at which point virus was also detected in the brain, thus bringing us back to the dengue neurology study [13••] with which this review began.

Conclusion

There is still much to learn about the pathogenesis of DHF. With the global spread of dengue virus infection, we also need to understand more about the correlates of disease severity and to relate these to host as well as virus-determined factors.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment and control. Geneva: WHO; 1986.

2 World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment and control, 2nd ed. Geneva: WHO; 1997.

3 Sumarmo , Wulur H, Jahja E, *et al*. Encephalopathy associated with dengue infection. Lancet 1978; 1:449–450.

4 Kho LK, Sumarmo , Wulur H, *et al*. Dengue haemorrhagic fever accompanied by encephalopathy in Jakarta. SEA J Trop Med Publ Health 1981; 12:583–590.

5 Nimmannitya S, Thisyakorn U, Hemsrichart V. Dengue haemorrhagic fever with unusual manifestations. SEA J Trop Med Publ Health 1987; 18:398–406.

6 Jirapinyo P, Treetrakarn A, Vajaradul A, Suvatte V. Dengue haemorrhagic fever: a case report with acute hepatic failure, protracted hypocalcemia, hyperamylasemia and an enlargement of the pancreas. J Med Assoc Thai 1988; 71:528–532.

7 Hendarto SK, Hadinegoro SR. Dengue encephalopathy. Acta Paed Jap 1992; 34:350–357.

8 Lum LCS, Lam SK, George R, Devi S. Fulminant hepatitis in dengue infection. SEA J Trop Med Publ Health 1993; 24:467–471.

9 Patey O, Ollivaud J, Breuil J, Lafaix C. Unusual neurologic manifestations occurring during dengue fever infection. Am J Trop Med Hyg 1993; 48:793–802.

10 Lum LCS, Lam SK, Choy S, *et al*. Dengue encephalitis: a true entity? Am J Trop Med Int Health 1996; 54:256–259.

11 Hommel D, Talarmin A, Deubel V, *et al*. Dengue encephalitis in French Guiana. Res Virol 1998; 149:235–238.

12 Mohan B, Patwari AK, Anand VK. Hepatic dysfunction in childhood dengue infection. J Trop Pediatr 2000; 46:40–43.

13 •• Solomon T, Dung NM, Vaughn DW, *et al*. Neurological manifestations of dengue infection. Lancet 2000; 355:1053–1059.
This is a unique prospective study of neurological dengue in southern Vietnam and includes a description of the clinical features seen in a group of 21 patients. There is even an attempt to include enough virology to suggest that the viruses isolated from these patients are from the same lineage as the viruses from patients with DHF in the same season.

14 Solomon T, Dung NM, Kneen R, *et al*. Japanese encephalitis. J Neurol, Neurosurg Psychiatry 2000; 68:405–415.

15 Innis BL. Japanese encephalitis. In: Exotic viral infections. Porterfield JS (editor). London: Chapman and Hall; 1995. pp. 147–174.

16 •• Rosen L, Drouet M-T, Deubel V. Detection of dengue virus RNA by reverse transcription–polymerase chain reaction in the liver and lymphoid organs but not in the brain in fatal human infection. Am J Trop Med Hyg 1999; 61:720–724.
The use of RT–PCR to determine the presence of dengue virus RNA in autopsy tissue is presented with careful attention to detail to convince the reader that the methods used have been optimised to give a high sensitivity. Negative results are therefore all the more convincing.

17 Rosen L, Khin M-M, Tin-U . Recovery of virus from the liver of children with fatal dengue: reflections on the pathogenesis of the disease and its possible analogy with that of yellow fever. Res Virol 1989; 140:351–360.

18 Kangwanpong D, Bhamarapravati N, Lucia HL. Diagnosing dengue virus infection in archived autopsy tissues by means of the in-situ PCR method: a case report. Clin Diagn Virol 1995; 3:165–172.

19 • Murgue B, Deparis X, Chungue E, *et al*. Dengue: an evaluation of dengue severity in French Polynesia based on an analysis of 403 laboratory-confirmed cases. Trop Med Int Health 1999; 4:765–773.
Although designed to test the validity of the WHO classification, this retrospective study provides much interesting data on clinical and laboratory correlates of disease severity.

20 Rigau-Perez JG, Bonilla GL. An evaluation of modified case definitions for the detection of dengue haemorrhagic fever. Puerto Rico Association of Epidemiologists. P R Health Sci J 1999; 18:347–352.

21 Gubler DJ, Clark GG. Dengue/dengue haemorrhagic fever: the emergence of a global health problem. Emerg Infect Dis 1995; 1:55–57.

22 Rico-Hesse R, Harrison LM, Salas RA, *et al*. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. Virology 1997; 230:244–251.

23 • Leitmeyer KC, Vaughn DW, Watts DM, *et al*. Dengue virus structural differences that correlate with pathogenesis. J Virol 1999; 73:4738–4747.
A massive effort in sequencing the complete genomes of a number of Southeast Asian and American strains of DEN-2. Even more remarkable is the fact that sequencing was performed on viraemic primary specimens rather than on passaged virus.

24 Sangkawibha N, Rojanasuphot S, Ahandrik S, *et al*. Risk factors in dengue shock syndrome: a prospective epidemiological study in Rayong, Thailand. Am J Epidemiol 1984; 20:653–669.

25 •• Watts DM, Porter KR, Putvatana P, *et al*. Failure of secondary infection with American genotype dengue 2 to cause dengue haemorrhagic fever. Lancet 1999; 354:1431–1434.
A prospective epidemiological study covering the period from 1990 through the epidemic of 1995 in Peru. The sequence of infection DEN-1 to DEN-2 was reminiscent of the Thai study a decade earlier. However, in Peru, this sequence of infection did not give rise to DHF.

26 • 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; 61:412–419.
Preliminary data from this Indonesian study show that sequential DEN-1 to DEN-2 infections did not give rise to DHF/DSS, but that the sequence DEN-2 to DEN-1 did result in DHF/DSS in 7.3% of these children.

27 Murgue B, Roche C, Chungue E, Deparis X. Prospective study of the duration and magnitude of viraemia in children hospitalised during the 1996–1997 dengue-2 outbreak in French Polynesia. J Med Virol 2000; 60:432–438.

28 •• 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.
The effort put into this study has been tremendous. It addresses many important questions regarding virus load and humoral responses in the pathogenesis of DHF. The study sample is large and is not limited to a single dengue virus serotype.

29 Rothman AL, Ennis FA. Immunopathogenesis of dengue haemorrhagic fever. Virology 1999; 257:1–6.

30 • Green S, Vaughn DW, Kalayanarooj S, *et al*. Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. J Infect Dis 1999; 179:755–762.
A nicely designed study with clear graphic representations of the associations of various mediators with markers of disease severity.

31 Ferguson N, Anderson R, Gupta S. The effect of antibody-dependent enhancement on the transmission dynamics and persistence of multiple-strain pathogens. Proc Natl Acad Sci USA 1999; 96:790–794.

32 Ferguson NM, Donnelly CA, Anderson RM. Transmission dynamics and epidemiology of dengue: insights from age-stratified sero-prevalence surveys. Phil Trans R Soc Lond B Biol Sci 1999; 354:757–768.

33 • Velzing J, Groen J, Drouet MT, *et al*. Induction of protective immunity against dengue virus type 2: comparison of candidate live attenuated and recombinant vaccines. Vaccine 1999; 17:1312–1320.
An important study of humoral immune responses in cynomolgus monkeys. Using monkeys is always an expensive exercise, and this and other (ethical) considerations dictate that the numbers of animals used remain too small to be useful for meaningful generalisations.

34 •• An J, Kimura-Kuroda J, Hirabayashi Y, Yasui K. Development of a novel mouse model for dengue virus infection. Virology 1999; 263:70–77.
This paper is the highlight of the year under review. A brilliant attempt to create an animal model that mimics human disease. This mouse model will surely be the means by which the long awaited breakthroughs into the mysteries of DHF pathogenesis will become possible.

35 Marianneau P, Megret F, Olivier R, *et al*. Dengue 1 virus binding to human hepatoma HepG2 and simian Vero cell surface differs. J Gen Virol 1996; 77:2547–2554.

36 Marianneau P, Cardono A, Edelman L, *et al*. Dengue virus replication in human hepatoma cells activates NF-κB which in turn reduces apoptotic cell death. J Virol 1997; 71:3244–3249.