INSTITUT PASTEUR

Microbes and Infection 12 (2010) 113–118

www.elsevier.com/locate/micinf

Review

Severe dengue: questioning the paradigm

Bernadette Murgue*

Institut National de la Santé et de la Recherche Médicale (INSERM), Institut de Maladies Infectieuses, 101 rue de Tolbiac, 75013 Paris, France

Received 6 March 2009; accepted 10 November 2009
Available online 17 November 2009

Abstract

Severe dengue has been recognised for more than 200 years, but attempts to define, categorize and explain the condition have hotly contested for more than four decades. Resolution of this controversy may provide new insights for the management of patients.

Keywords: Dengue; Clinical; Pathogenesis; Risk factors

1. Introduction

There is a common misconception that severe forms of dengue first appeared in 1950s in southeast Asia. While it is true that the syndrome became a serious public health problem in that period, it was not a new phenomenon; significant mortality associated with haemorrhagic symptoms had been described in the earliest epidemic of dengue-like disease on record, in Philadelphia in 1780, and in later epidemics on the Swahili coast of East Africa and in Australia [1]. Even in Europe, at least 1000 people died in 1927–1928 in a massive epidemic—1,000,000 cases—in refugee camps in Greece [2].

Severe dengue—commonly referred to as ''Dengue Haemorrhagic Fever (DHF)'' to distinguish it from ''classic'' dengue fever (DF)—is characterized by an increase of vascular permeability that can lead to life-threatening hypovolemic shock. The mechanisms that give rise to this condition have been debated for decades, but remain unresolved. There are four distinct serotypes of the virus, designated DEN-1 to DEN-4, and a widely held but hotly contested hypothesis is that after a ''primary'' infection with one of these serotypes, ''secondary'' infections by one or more of the others can precipitate a process referred to as ''antibody dependant enhancement'' (ADE). Confirmation of this hypothesis is hampered by the absence of a reliable animal model. An associated controversy is the validity of graded sets of criteria to categorize severity that are recommended by the World Health Organization (WHO). Both issues are of prime importance for the management and treatment of patients, and to future acceptance of dengue vaccines.

In the years after World War II, rapid urbanization coupled with enormous infestations of the highly competent peridomestic vector, *Aedes aegypti* L., led to an explosive increase in the prevalence and incidence of dengue in south-east Asia. A simple explanation for the «new» syndrome is the «iceberg effect»; the escalating numbers of ''classic'' infections led to an increased *awareness* of the relatively rare manifestations that had been first observed more than two centuries earlier. Moreover, as all four serologically distinct dengue viruses were in circulation—in essence four separate diseases—the population was subject to an increasing *frequency* of epidemic transmission. Nevertheless, the concept that DHF had emerged as a new form of the disease gained rapid acceptance in the medical world [3].

In the mid 70's, the WHO [4] proposed a clinical classification of dengue severity to assist in the diagnosis and management of patients, and to monitor the incidence of the disease. According to this classification, dengue could manifest in two ways:

1. Dengue Fever (DF), a relatively benign disease, with an acute phase of 3–7 days and a combination of symptoms

* Tel.: +33 0 1 44 23 63 67.
E-mail address: bernadette.murgue@inserm.fr

1286-4579/$ - see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.micinf.2009.11.006

that could include sudden onset of high fever, headache, myalgia, arthralgia, rash and moderate haemorrhage, followed by a prolonged convalescence but without serious sequelae;
2. Dengue Haemorrhagic Fever (DHF), a severe, sometimes life-threatening disease, characterized by plasma leakage due to an increase of vascular permeability. This was defined by haemo-concentration with a 20% or greater increase of haematocrit and a platelet count below 100,000/mm^3.

DHF was assigned four grades of severity. In grades 1 and 2 there were no signs of circulatory failure whereas grades 3 and 4, termed ''Dengue Haemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS)'', were characterized by circulatory failure and hypovolemic shock that could be fatal in 5–15% of cases.

2. DHF and the sequential infection theory

Early observations in Bangkok, Thailand, indicated that, with the exception of infants less than 1-year of age, the majority of patients with DHF had experienced one or more previous dengue infections; this led to the sequential infection hypothesis. A process termed ''Antibody Dependent Enhancement'' (ADE), demonstrated by *in vitro* experiments, was proposed: primary infections give rise to non-neutralizing but enhancing antibodies which, in a subsequent infection (by another serotype) enhance the entry of virus into Fc-γ receptor-positive cells such as monocytes and phagocytes. This results in an increase in virus replication and the release of mediators of vascular permeability that give rise to the shock syndrome [5]. An additional hypothesis, termed ''aberrant T-cell response'', was also proposed [6] to explain disease severity during secondary dengue infection: the reactivation of serotype cross-reactive memory T cells gives rise to abnormal T-cell activation and cytokine release or apoptosis. The two mechanisms were not considered to be mutually exclusive. By the mid-1970s, it was widely accepted that severe dengue, as described by the WHO criteria, was fully attributable to sequential dengue infections.

In vitro enhancing activity has also been reported for sequential flavivirus infections, but, except for an inconclusive study with monkeys, has never been demonstrated *in vivo* [7].

3. Questioning the paradigm

In SE Asia, confirmation of the ADE controversy was hampered by the high incidence of transmission, particularly in urban areas, but contrary evidence was apparent in several other outbreaks. In 1972, for example, an epidemic of DEN-2 occurred on Niue, a remote island in the South Pacific where there was no evidence of any dengue transmission for at least 25 years [8]. About 90% of the population (4600) in all age groups were affected. A number of patients fitted the DHF definition, with 12 fatal cases that included children less than 15 years of age; there was no doubt that these were primary cases. Kuberski et al. [9] made similar observations during a DEN-1 outbreak in Fiji in 1975. They found no significant difference in the incidence of haemorrhage and other clinical symptoms in primary vs. secondary infections. In a Bangkok study, about ten percent of children with DHF/DSS, all four years old or more, were primary infections, two of which were fatal [10]. Lastly a retrospective study of the 1927–1928 DEN-1 epidemic in Greece confirmed that fatal cases had occurred in many patients with primary infections [11].

In the light of these observations, Rosen questioned the justification for separating dengue into two clinical entities—benign and severe—and the validity of the WHO definition of DHF He suggested that, as with other infectious diseases, there was a continuum from non-clinical to fatal cases, and that haemorrhage and shock were not necessarily linked to the same pathogenic mechanism [9]. Moreover, an intensive review of reports on the involvement of specific organs, revealed that unusual haemorrhage, severe liver and neurologic perturbations had been described for both DF and DHF [12].

Despite all this evidence supporters of the ADE concept continued to maintain that severe cases, defined as DHF, were the result of secondary infection. As a result, it was feared that a monovalent vaccine could induce enhancing antibodies, with serious consequences, and a tetravalent vaccine might carry the same risk if response to one or more serotypes were incomplete.

In Tahiti, Murgue et al [13] examined risk factors for DHF after two consecutive epidemics – DEN-3 in 1989 and DEN-2 in 1996. Their study was based on 401 children hospitalized with dengue with ages ranging from less than 1 year (one patient) to 17 years. Ten of these cases were fatal. They used the WHO classification and a range of other clinical and biological criteria to select 50 of the most severe cases. Seventeen of these (34%) did not fulfil the requirements for DHF (severe dengue) because there was no plasma leakage, yet these included six of the ten fatal cases, with hepatic involvement (transaminases >20 times), severe thrombocytopenia ($<20{,}000$), severe haemorrhage and shock. They also reviewed cases that fitted the DHF definition; seven of 49 (14.5%) were primary infections, of which all but one were more than one year old. They concluded that (i) many severe cases did not fit the WHO definition of DHF and (ii) a significant portion of DHF cases were primary infections. They also questioned the relevance of the WHO classification in the management of DHF cases because the main criteria (haemoconcentration) could only be assessed in retrospect. Several subsequent studies in Southeast Asia, Central and North America have confirmed and extended these conclusions.

4. The inadequacies of the WHO classification

Irrespective of classification as DF (defined by absence of haemoconcentration and/or thrombocytopenia) or DHF, severe dengue is associated with a range of manifestations including haemorrhage and/or thrombocytopenia [13–16], hepatic alterations [13,17,18], central nervous system manifestations

[13,18,19] and shock syndrome [14–16]. Acute distress respiratory syndrome [20], heart disease [21], renal insufficiency [22], pancreatitis [23], hemophagocytosis, spontaneous spleen rupture have also been reported, as well as serious sequelae [24–26].

In addition, although hypovolemic shock associated with plasma leakage is an obvious manifestation of severe disease, plasma leakage can occur without thrombocytopenia or haemorrhage [14,16]. Moreover, several studies have noted the difficulty of assessing capillary leakage during the acute phase of illness, when clinical signs of effusion are absent, and when, in the absence of a pre-infection value, the haematocrit appears within a ''normal range'' [13]. In such cases, diagnosis can only be made restrospectively, i.e., when the haematocrit has stabilized, and is therefore of marginal value in the management of patients. Lastly, in consequence of the WHO classification, there is a tendency for physicians to classify all severe cases as DHF [13–15]. This results in misleading estimates of the incidence of severe cases and additional confusion over the pathogenesis of the disease.

From the above, as stated by Deen [27] and Setiati [28], it is evident that the WHO definitions of DF and DHF are inadequate and misleading. The best that can be said is that, as with many other viral infections, infection can present with a wide spectrum of severity, from asymptomatic to serious and sometimes fatal. An alternate approach could be to classify cases as following:

1. Severe vs. non-severe
2. Severe cases would cover the full range of severe manifestations, including shock with or without plasma leakage, liver involvement, central nervous system involvement, haemorrhage, thrombocytopenia, etc.
3. The definition of shock due to plasma leakage would be (i) simple and practical for the diagnosis and the management of the patients, (ii) independent of thrombocytopenia or bleeding; (iii) suitable for use in countries where dengue is endemic or epidemic.

5. Persistence of the sequential infection hypothesis

In the past decade, following the reports of Barnes & Rosen [8] and Murgue et al. [13], a number of authors have reported plasma leakage in primary infections [15,16,19,29,30]. Moreover, in Nicaragua, Harris et al. [15] observed that 50% of confirmed dengue infections in 1-year olds and 90% in 3-year olds were secondary infections but were not associated with plasma leakage or shock, and the same was true for adults. Lastly, in a prospective study conducted in Thailand Laoprasopwattana et al. evaluated enhancing activity in the plasma of 60 children during pre-secondary dengue infection [31]. The majority of samples enhanced dengue infection of human cells *in vitro*, but this enhancing activity did not predict clinical severity in subsequent infections. Despite this wealth of epidemiological and laboratory evidence, the hypotheses of sequential infection hypothesis and the hypothesis of enhancing antibodies remain persists, and indeed is widely accepted. To resolve this controversy, and despite widen evidence that plasma leakage occur during both primary and secondary infections, it is essential to demonstrate that secondary infection does not augment the risk of this phenomenon. The absence of an appropriate animal model is a major impediment to resolution of this question.

Inoculation of the rhesus monkey, *Macaca mulatto* with dengue virus (DENV) induces viraemia, neutralizing antibodies and changes in haematological parameters, but infections are asymptomatic so these animals are not suitable models for investigation of pathogenesis [32]. As an alternative, A/J SCID mice reconstituted with human cells were infected with DENV cultured in mosquito cell lines or with mouse brain adapted strains. There was no alteration of vascular permeability, but paralysis, in some cases associated with haemostatic or haematopoietic abnormalities [33,34] and liver involvement was observed. However, NOD/SCID mice xenografted with CD34+ cells, and infected with a dengue 2 serotype that gives high titres *in vitro* in human dendritic cells, induced erythaema and thrombocytopenia but no paralysis [35].

In another study, mice lacking interferon receptors were infected with an initially non-mouse adapted dengue 2 strain that had been alternately passaged in mosquito cells and in mice [36]. There were no symptoms of paralysis, but there were signs of increased vascular permeability, producing significant levels of TNF-α. The same mice infected with the parental DENV develop paralysis and had normal vascular permeability. When inoculated subcutaneously with the passaged dengue 2 strain, infection was primarly in macrophages and dendritic cells during the first week of infection, a cell tropism that has also been reported in humans [37]. Virus was detected in lymph nodes, spleen, bone marrow and circulating white blood cells, but not in the liver.

In vitro and *in vivo* studies indicate that when DENV is injected into the dermis, the primary targets for infection are the skin immature dendritic cells (DCs) that are normal residents of the skin [38]. Infection is not altered by a DENV-enhancing immune serum [38,39]. The attachment of DENV to DCs is through the expression of the C-type lectin: DC-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing non-integrin (DC-SIGN or CD209) [39]. Early interactions between mosquito-cell-derived DENV and DCs may be crucial for productive infection [40], for transporting viral antigens to secondary lymphoid organs and for developing anti-viral immunity. *In vitro* studies have demonstrated that a broad array of other enveloped viruses exploit DC-SIGN, or more generally C-type lectin for infection [41] (Lazach PY in Methods Mol Biol, 2007; 379:51-68).

Replication of DENV has been confirmed by immunohistochemistry and *in situ* hybridization in tissue specimens from patients with serologically or virologically confirmed dengue infections and plasma leakage.

Viral antigens were detected in the liver (Kupffer cells and sinusoidal endothelial cells), in the spleen (macrophages, multinucleated cells and reactive lymphoid cells) as well as in

the kidney tubules, in the lung and blood clot samples. However, viral RNA was only demonstrated in cells in the spleen and blood clot samples (macrophages, reactive splenic lymphoid cells), peripheral lymphocytes and peripheral blood monocytes [42]

After attachment and viral replication, complex interactions between viral factors, host genetics and immunological response via chemical mediators probably contribute to the development of severe manifestations (including plasma leakage), which may be associated with enhanced viraemia [43,44]. Phylogenetic studies have suggested a possible association between specific genotypes within a serotype, and clinical outcome of the disease. The best example is the Southeast Asian genotype of DEN-2 which has been identified in outbreaks with plasma leakage and/or hypovolemic shock. This genotype has superceded the less virulent native or autochthonous genotypes in the Americas [45]. Determinants of higher virulence in the Southeast Asian genotype may be related to specific regions of the viral genome that give a higher probability of infection, transmission and displacement. Other studies have demonstrated differences in nucleotides or amino-acid sequences of the viral genome, mostly on the *E* gene DEN-2 virus but also on complete genome sequences. This suggests that structural differences may be involved in disease severity [46]. It is not clear, however, whether some strains are virulent because they grow well *in vivo*, because they are highly cytopathic, or because they induce high levels of cytokines.

On the other hand, Roche et al. [47] observed no association between amino acid substitutions and disease severity among nine isolates of DEN-3 virus, six of which were derived from cases with severe manifestations (without or with plasma leakage) including both primary and secondary infections. These data suggest a more complex mechanism involving the interaction of host and other factors. Indeed, clinical isolates of DENV had an *in vitro* inhibitory effect on the growth of human haematopoietic progenitor cells obtained from fresh cord blood samples, dependent upon the dengue viral strain [48] but also on the cord blood sample used (Murgue, unpublished data). These observations may indicate (i) a differential capacity of DENV strains to infect DC-SIGN-expressing cells or others and thus a differential infectivity [40] and/or (ii) evidence of genetic heterogeneity of DENV receptors such as DC-SIGN [49] and/or (iii) a differential release of chemical mediators.

Several *in vitro* studies have suggested that the dysfunction of vascular endothelial cells during plasma leakage is related to the production of cytokines. *In vivo,* an increase of T cell activation markers and of various cytokines [50] and adhesion molecules [51–54] have been observed in the sera of dengue patients with plasma leakage. However, it is not clear whether these factors are inducers or markers of the increase of vascular permeability.

Overproduction of soluble gelatinolytic matrix metalloproteinase (MMP)-9, and to a lesser extent of MMP-2 in a viral dose-dependant and antibody-independent manner, has been demonstrated after DENV infection of immature DCs [55]. *In vitro*, these compounds enhanced endothelial permeability of primary umbilical vascular endothelial cell monolayers, but permeability was markedly reduced by a specific anti MMP-9 antibody. These findings were confirmed *in vivo*, using a mouse vascular leakage model. The enhancement of permeability was associated with a loss of expression of the platelet endothelial adhesion molecule-1 and vascular endothelial-cadherin cell adhesion molecule and, a redistribution of F-actin fibres. The production of gelatinolytic MMP could be activated by soluble factors such as IL-6, IL-8, TNF-α, TGF-β, VEGF and VCAM-1, induced in response to dengue infection. These results could prove useful in therapeutic approaches to the treatment of dengue-induced vascular leakage.

For physicians, the issue of overwhelming importance is how to anticipate the likelihood of the development of plasma leakage and subsequent complications. As already mentioned, haemoconcentration can fulfil this role but in practice is of limited value. A number of other predictive factors have been suggested but investigation have been plagued by lack of sufficient samples during the course of the disease. However, a few studies have indicated elevated levels of soluble thrombomodulin, sICAM-1, sVCAM-1 [52], and circulating endothelial cells in the early febrile stage of patients who subsequently presented signs of plasma leakage (with or without a shock syndrome). A coincident increase of sICAM-1, sVCAM-1 and circulating endothelial cells has been reported in patients with plasma leakage. Furthermore, sVCAM-1 might be able to discriminate, between patients with plasma leakage, those who will develop a shock syndrome, without regard to previous infection [52].

6. Conclusion

Physicians confronted with cases of severe dengue face a dilemma; several authors have challenged the WHO classification because it fails to describe the overall range of severe manifestations, and it is clear that the issue requires a new approach.

The development of vaccines has been hampered by the sequential infection hypothesis, which a number of clinical studies have showed to be invalid. In recent years, new perspectives on the issue of the pathogenesis have been explored, but have not resolved the entire question. Recent studies are cause for optimism that it may be possible to predict the likely progression of the disease. The resolution of all these issues will require funding for a coordinated multidisciplinary effort, including experimentation of promising therapeutic drugs in clinical trials.

References

[1] D.J. Gubler, G. Kuno, Dengue and dengue hemorrhagic fever. CAB International, Wallingford, Oxon, UK, New York, 1997.

[2] G. Papaevangelou, S.B. Halstead, Infections with two dengue viruses in Greece in the 20th century. Did dengue hemorrhagic fever occur in the 1928 epidemic? The Journal of Tropical Medicine and Hygiene 80 (1977) 46–51.

[3] W.M. Hammon, A. Rudnick, G. Sather, K.D. Rogers, L.J. Morse, New hemorrhagic fevers of children in the Philippines and Thailand. Transactions of the Association of American Physicians 73 (1960) 140–155.
[4] WHO (Ed.), Technical guidelines for diagnosis, treatment and control, 1997.
[5] S.B. Halstead, E.J. O'Rourke, Antibody-enhanced dengue virus infection in primate leukocytes. Nature 265 (1977) 739–741.
[6] I. Kurane, B.L. Innis, S. Nimmannitya, A. Nisalak, A.L. Rothman, P.G. Livingston, J. Janus, F.A. Ennis, Human immune responses to dengue viruses. The Southeast Asian Journal of Tropical Medicine and Public Health 21 (1990) 658–662.
[7] S.B. Halstead, J. Casals, H. Shotwell, N. Palumbo, Studies on the immunization of monkeys against dengue. I. Protection derived from single and sequential virus infections. The American Journal of Tropical Medicine and Hygiene 22 (1973) 365–374.
[8] W.J. Barnes, L. Rosen, Fatal hemorrhagic disease and shock associated with primary dengue infection on a Pacific island. The American Journal of Tropical Medicine and Hygiene 23 (1974) 495–506.
[9] T. Kuberski, L. Rosen, D. Reed, J. Mataika, Clinical and laboratory observations on patients with primary and secondary dengue type 1 infections with hemorrhagic manifestations in Fiji. The American Journal of Tropical Medicine and Hygiene 26 (1977) 775–783.
[10] R.M. Scott, S. Nimmannitya, W.H. Bancroft, P. Mansuwan, Shock syndrome in primary dengue infections. The American Journal of Tropical Medicine and Hygiene 25 (1976) 866–874.
[11] L. Rosen, Dengue in Greece in 1927 and 1928 and the pathogenesis of dengue hemorrhagic fever: new data and a different conclusion. The American Journal of Tropical Medicine and Hygiene 35 (1986) 642–653.
[12] R. George, L. Lum, Clinical spectrum of dengue infection. in: D. Guubler, G. Kuno (Eds.), Dengue and dengue hemorrhagic fever. CAB International, Wallingford, UK, 1997, pp. 89–114.
[13] B. Murgue, X. Deparis, E. Chungue, O. Cassar, C. Roche, Dengue: an evaluation of dengue severity in French Polynesia based on an analysis of 403 laboratory-confirmed cases. Trop Med Int Health 4 (1999) 765–773.
[14] C.X. Phuong, N.T. Nhan, R. Kneen, P.T. Thuy, C. van Thien, N.T. Nga, T. T. Thuy, T. Solomon, K. Stepniewska, B. Wills, Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: is the world health organization classification system helpful? The American Journal of Tropical Medicine and Hygiene 70 (2004) 172–179.
[15] E. Harris, E. Videa, L. Perez, E. Sandoval, Y. Tellez, M.L. Perez, R. Cuadra, J. Rocha, W. Idiaquez, R.E. Alonso, M.A. Delgado, L.A. Campo, F. Acevedo, A. Gonzalez, J.J. Amador, A. Balmaseda, Clinical, epidemiologic, and virologic features of dengue in the 1998 epidemic in Nicaragua. The American Journal of Tropical Medicine and Hygiene 63 (2000) 5–11.
[16] A. Balmaseda, S.N. Hammond, M.A. Perez, R. Cuadra, S. Solano, J. Rocha, W. Idiaquez, E. Harris, Short report: assessment of the World Health Organization scheme for classification of dengue severity in Nicaragua. The American Journal of Tropical Medicine and Hygiene 73 (2005) 1059–1062.
[17] L.J. Souza, J.G. Alves, R.M. Nogueira, C. Gicovate Neto, D.A. Bastos, E.W. Siqueira, J.T. Souto Filho, A. Cezario Tde, C.E. Soares, C. Carneiro Rda, Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1,585 cases. The Brazilian journal of infectious diseases 8 (2004) 156–163.
[18] R.B. Domingues, G.W. Kuster, F.L. Onuki-Castro, V.A. Souza, J.E. Levi, C.S. Pannuti, Involvement of the central nervous system in patients with dengue virus infection. Journal of the neurological sciences (2007).
[19] T. Solomon, N.M. Dung, D.W. Vaughn, R. Kneen, L.T. Thao, B. Raengsakulrach, H.T. Loan, N.P. Day, J. Farrar, K.S. Myint, M.J. Warrell, W.S. James, A. Nisalak, N.J. White, Neurological manifestations of dengue infection. Lancet 355 (2000) 1053–1059.
[20] C.C. Wang, S.F. Liu, S.C. Liao, I.K. Lee, J.W. Liu, A.S. Lin, C.C. Wu, Y. H. Chung, M.C. Lin, Acute respiratory failure in adult patients with dengue virus infection. The American Journal of Tropical Medicine and Hygiene 77 (2007) 151–158.
[21] I. Obeyesekere, Y. Hermon, Arbovirus heart disease: myocarditis and cardiomyopathy following dengue and chikungunya fever – a follow-up study. American heart journal 85 (1973) 186–194.
[22] W.J. Wiersinga, C.G. Scheepstra, J.S. Kasanardjo, P.J. de Vries, H. Zaaijer, S.E. Geerlings, Dengue fever-induced hemolytic uremic syndrome. Clinical infectious diseases 43 (2006) 800–801.
[23] T. Derycke, P. Levy, B. Genelle, P. Ruszniewski, C. Merzeau. Acute pancreatitis secondary to dengue, Gastroenterologie Clinique et Biologique 29 (2005) 85–86.
[24] D.P. Chan, S.C. Teoh, C.S. Tan, G.K. Nah, R. Rajagopalan, M.K. Prabhakaragupta, C.K. Chee, T.H. Lim, K.Y. Goh, Ophthalmic complications of dengue. Emerging Infectious Diseases 12 (2006) 285–289.
[25] S.A. de Oliveira, L.A. Camacho, L.R. Bettini, D.G. Fernandes, N.A. Gouvea, R.A. Barros, S. Setubal, M.M. Siqueira. The joint manifestations of exanthematous viroses, Revista da Sociedade Brasileira de Medicina Tropical 32 (1999) 125–130.
[26] R.C. Seet, A.M. Quek, E.C. Lim, Post-infectious fatigue syndrome in dengue infection. Journal of clinical virology 38 (2007) 1–6.
[27] J.L. Deen, E. Harris, B. Wills, A. Balmaseda, S.N. Hammond, C. Rocha, N.M. Dung, N.T. Hung, T.T. Hien, J.J. Farrar, The WHO dengue classification and case definitions: time for a reassessment. Lancet 368 (2006) 170–173.
[28] T.E. Setiati, A.T. Mairuhu, P. Koraka, M. Supriatna, M.R. Mac Gillavry, D.P. Brandjes, A.D. Osterhaus, J.W. van der Meer, E.C. van Gorp, A. Soemantri, Dengue disease severity in Indonesian children: an evaluation of the World Health Organization classification system. BMC Infectious Diseases 7 (2007) 22.
[29] A. Suwandono, H. Kosasih, Nurhayati, R. Kusriastuti, S. Harun, C. Ma'roef, S. Wuryadi, B. Herianto, D. Yuwono, K.R. Porter, C.G. Beckett, P.J. Blair, Four dengue virus serotypes found circulating during an outbreak of dengue fever and dengue haemorrhagic fever in Jakarta, Indonesia, during 2004. Transactions of the Royal Society of Tropical Medicine and Hygiene 100 (2006) 855–862.
[30] R.M. Nogueira, M.P. Miagostovich, R.V. Cunha, S.M. Zagne, F.P. Gomes, A.F. Nicol, J.C. Coelho, H.G. Schatzmayr, Fatal primary dengue infections in Brazil. Transactions of the Royal Society of Tropical Medicine and Hygiene 93 (1999) 418.
[31] K. Laoprasopwattana, D.H. Libraty, T.P. Endy, A. Nisalak, S. Chunsuttiwat, D.W. Vaughn, G. Reed, F.A. Ennis, A.L. Rothman, S. Green, Dengue Virus (DV) enhancing antibody activity in preillness plasma does not predict subsequent disease severity or viremia in secondary DV infection. The Journal of Infectious Diseases 192 (2005) 510–519.
[32] W. Sun, A. Nisalak, M. Gettayacamin, K.H. Eckels, J.R. Putnak, D.W. Vaughn, B.L. Innis, S.J. Thomas, T.P. Endy, Protection of Rhesus monkeys against dengue virus challenge after tetravalent live attenuated dengue virus vaccination. The Journal of Infectious Diseases 193 (2006) 1658–1665.
[33] K.J. Huang, S.Y. Li, S.C. Chen, H.S. Liu, Y.S. Lin, T.M. Yeh, C.C. Liu, H.Y. Lei, Manifestation of thrombocytopenia in dengue-2-virus-infected mice. The Journal of General Virology 81 (2000) 2177–2182.
[34] A.J. Johnson, J.T. Roehrig, New mouse model for dengue virus vaccine testing. Journal of Virology 73 (1999) 783–786.
[35] D.A. Bente, M.W. Melkus, J.V. Garcia, R. Rico-Hesse, Dengue fever in humanized NOD/SCID mice. Journal of Virology 79 (2005) 13797–13799.
[36] S. Shresta, K.L. Sharar, D.M. Prigozhin, P.R. Beatty, E. Harris, Murine model for dengue virus-induced lethal disease with increased vascular permeability. Journal of Virology 80 (2006) 10208–10217.
[37] J.L. Kyle, P.R. Beatty, E. Harris, Dengue virus infects macrophages and dendritic cells in a mouse model of infection. The Journal of Infectious Diseases 195 (2007) 1808–1817.
[38] S.J. Wu, G. Grouard-Vogel, W. Sun, J.R. Mascola, E. Brachtel, R. Putvatana, M.K. Louder, L. Filgueira, M.A. Marovich, H.K. Wong, A. Blauvelt, G.S. Murphy, M.L. Robb, B.L. Innes, D.L. Birx, C.G. Hayes, S. S. Frankel, Human skin Langerhans cells are targets of dengue virus infection. Nature Medicine 6 (2000) 816–820.
[39] B. Tassaneetrithep, T.H. Burgess, A. Granelli-Piperno, C. Trumpfheller, J. Finke, W. Sun, M.A. Eller, K. Pattanapanyasat, S. Sarasombath, D.L.

Birx, R.M. Steinman, S. Schlesinger, M.A. Marovich, DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. The Journal of Experimental Medicine 197 (2003) 823–829.

[40] E. Navarro-Sanchez, R. Altmeyer, A. Amara, O. Schwartz, F. Fieschi, J.L. Virelizier, F. Arenzana-Seisdedos, P. Despres, Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. EMBO Reports 4 (2003) 723–728.

[41] A. Cambi, M. Koopman, C.G. Figdor, How C-type lectins detect pathogens. Cell Microbiol 7 (2005) 481–488.

[42] K. Jessie, M.Y. Fong, S. Devi, S.K. Lam, K.T. Wong, Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. The Journal of Infectious Diseases 189 (2004) 1411–1418.

[43] B. Murgue, C. Roche, E. Chungue, X. Deparis, Prospective study of the duration and magnitude of viraemia in children hospitalised during the 1996–1997 dengue-2 outbreak in French Polynesia. Journal of Medical Virology 60 (2000) 432–438.

[44] D.H. Libraty, T.P. Endy, H.S. Houng, S. Green, S. Kalayanarooj, S. Suntayakorn, W. Chansiriwongs, D.W. Vaughn, A. Nisalak, F.A. Ennis, A.L. Rothman, Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. The Journal of Infectious Diseases 185 (2002) 1213–1221.

[45] R. Rico-Hesse, L.M. Harrison, R.A. Salas, D. Tovar, A. Nisalak, C. Ramos, J. Boshell, M.T. de Mesa, R.M. Nogueira, A.T. da Rosa, Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. Virology 230 (1997) 244–251.

[46] K.C. Leitmeyer, D.W. Vaughn, D.M. Watts, R. Salas, I. Villalobos, C. de, C. Ramos, R. Rico-Hesse, Dengue virus structural differences that correlate with pathogenesis. Journal of Virology 73 (1999) 4738–4747.

[47] C. Roche, O. Cassar, M. Laille, B. Murgue, Dengue-3 virus genomic differences that correlate with in vitro phenotype on a human cell line but not with disease severity. Microbes and infection/Institut Pasteur 9 (2007) 63–69.

[48] B. Murgue, O. Cassar, M. Guigon, E. Chungue, Dengue virus inhibits human hematopoietic progenitor growth in vitro. The Journal of Infectious Diseases 175 (1997) 1497–1501.

[49] A. Sakuntabhai, C. Turbpaiboon, I. Casademont, A. Chuansumrit, T. Lowhnoo, A. Kajaste-Rudnitski, S.M. Kalayanarooj, K. Tangnararatchakit, N. Tangthawornchaikul, S. Vasanawathana, W. Chaiyaratana, P.T. Yenchitsomanus, P. Suriyaphol, P. Avirutnan, K. Chokephaibulkit, F. Matsuda, S. Yoksan, Y. Jacob, G.M. Lathrop, P. Malasit, P. Despres, C. Julier, A variant in the CD209 promoter is associated with severity of dengue disease. Nature genetics 37 (2005) 507–513.

[50] I. Kurane, Dengue hemorrhagic fever with special emphasis on immunopathogenesis. Comparative immunology, microbiology and infectious diseases 30 (2007) 329–340.

[51] B. Murgue, O. Cassar, X. Deparis, Plasma concentrations of sVCAM-1 and severity of dengue infections. Journal of Medical Virology 65 (2001) 97–104.

[52] P. Koraka, B. Murgue, X. Deparis, E.C. Van Gorp, T.E. Setiati, A.D. Osterhaus, J. Groen, Elevation of soluble VCAM-1 plasma levels in children with acute dengue virus infection of varying severity. Journal of Medical Virology 72 (2004) 445–450.

[53] L.C. Chen, H.Y. Lei, C.C. Liu, S.C. Shiesh, S.H. Chen, H.S. Liu, Y.S. Lin, S.T. Wang, H.W. Shyu, T.M. Yeh, Correlation of serum levels of macrophage migration inhibitory factor with disease severity and clinical outcome in dengue patients. The American Journal of Tropical Medicine and Hygiene 74 (2006) 142–147.

[54] A. Srikiatkhachorn, C. Ajariyakhajorn, T.P. Endy, S. Kalayanarooj, D.H. Libraty, S. Green, F.A. Ennis, A.L. Rothman, Virus-induced decline in soluble vascular endothelial growth receptor 2 is associated with plasma leakage in dengue hemorrhagic Fever. Journal of Virology 81 (2007) 1592–1600.

[55] N. Luplertlop, D. Misse, D. Bray, V. Deleuze, J.P. Gonzalez, V. Leardkamolkarn, H. Yssel, F. Veas, Dengue-virus-infected dendritic cells trigger vascular leakage through metalloproteinase overproduction. EMBO Reports 7 (2006) 1176–1181.