The importance of hematopoietic progenitor cells in dengue

Jih-Jin Tsai, Li-Teh Liu, Ko Chang, Shu-Hui Wang, Hui-Mien Hsiao, Kristina B. Clark and Guey Chuen Perng

Ther Adv Hematol
(2011) 3(1) 59–71
DOI: 10.1177/2040620711417660

Abstract: Scientific investigations designed to better understand and assess the distinguishing clinical characteristics pave the way to a successful treatment for a disease. Since the peripheral blood is obtained easily, the most frequent type of investigation performed on infectious agents focuses on the hematological components of blood drawn from patients. Bone marrow aspirates, although somewhat more difficult to obtain, should be evaluated more frequently because they provide additional information, giving us a glimpse into the development of the disease. Understanding the distinct and unique changes in hematological components of the bone marrow induced by a particular pathogen or corresponding to a specific illness may be a valuable asset for the diagnosis and prognosis of disease. A good example of a pathogen that could be better evaluated with greater knowledge of the bone marrow is dengue, one of the most important public vector-borne human diseases. Owing to the multitude of clinical manifestations and the dynamic alterations of various blood components over time, this disease is one of the most difficult to prevent and treat in humans. Although large amounts of data have been generated in the literature, there remains a large gap between this information and its relevance for the purpose of patient care. While evaluating the cellular components in the circulated blood from ill patients provides us with valuable information about the pathogenesis of various pathogens, there are other players participating in the progression to disease. The goal of this review is to emphasize the importance of bone marrow hematopoietic progenitor cells in disease and to inspire other researchers to incorporate them into their investigations on dengue pathogenesis. It is anticipated that the knowledge derived from these investigations not only elicit original concepts on the pathogenesis of dengue but also foster a new way of thinking in terms of vaccine or therapeutic development to prevent and treat dengue.

Keywords: dengue fever, DHF, flavivirus, hematology disorder

Correspondence to:
Guey Chuen Perng
Department of Pathology and Laboratory Medicine, Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA 30322, USA,
gperng@emory.edu

Jih-Jin Tsai
Tropical Medicine Center, Kaohsiung Medical University Hospital, and Division of Infectious Diseases, Department of Internal Medicine, Medical University Hospital, Kaohsiung, Taiwan

Li-Teh Liu
Department of Medical Laboratory Science and Biotechnology, College of Medicine and Life Science, Chung-Hwa University of Medical Technology, Taiwan

Ko Chang
Tropical Medicine Center, Kaohsiung Medical University Hospital, Division of Infectious Diseases, Department of Internal Medicine, Medical University Hospital, and Department of Internal Medicine, Kaohsiung Municipal Hsaio-Kang Hospital, Kaohsiung, Taiwan

Shu-Hui Wang
Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

Hui-Mien Hsiao, Kristina B. Clark
Department of Pathology and Laboratory Medicine, Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA, USA

Dengue

Dengue has been recognized as one of the most important vector-borne human diseases. It has been mainly limited to the tropical and/or subtropical regions of the globe, but recently, due to factors such as the increased frequency of human migration, unplanned urban development, and ineffective vector control, the disease has penetrated into almost every corner of the planet [Guzman *et al.* 2010]. It is estimated that more than 100 countries are affected by dengue, and more than 100 million populations are at risk of the disease, having the potential to propagate outbreaks [WHO, 2009]. Therefore, dengue has become an imminent public health threat, even for places where the disease hardly ever surfaces, such as the US [Guzman *et al.* 2010; Morens and Fauci, 2008]. Currently, there is no commercial and US Food and Drug Administration (FDA)-approved vaccines or therapeutic drugs to prevent and treat dengue disease.

One of the key aspects of dengue is viremia, which occurs during the febrile stage of illness [Gubler *et al.* 1981]. Another characteristic is plasma leakage, which is associated with severe clinical manifestations, such as shock, and usually occurs at the time when fever abates and virus clearance approaches completion [Bethell *et al.* 2001; Srichaikul and Nimmannitya, 2000;

Nimmannitya, 1987]. Thus, hematological components have been investigated intensively in order to determine the unique clinical signature for dengue virus (DENV) illness in patients [Srichaikul and Nimmannitya, 2000]. Several hypotheses have been proposed to decipher the mechanisms involved in plasma leakage and thrombocytopenia, which correlate with the clinical disease [Halstead, 2007]. Readers who are interested in the general aspects of hematology in dengue are directed to some recent comprehensive review articles [Martina *et al.* 2009; Fink *et al.* 2006; Srichaikul and Nimmannitya, 2000; Nelson *et al.* 1966; Nelson and Bierman, 1964; Halstead, 1982]. The current review is specifically focused on the hematopoietic progenitor cell components that are relevant to the pathogenesis of dengue.

Clinical observations

Dengue is transmitted by *Aedes spp.* mosquitoes, carrying infectious DENV, which can be categorized into four distinct serotypes, DENV1, DENV2, DENV3, and DENV4. Each serotype is capable of inducing a full spectrum of disease symptoms in patients, although differences in virulence may exist [Murphy and Whitehead, 2011; Rico-Hesse, 2009; WHO, 2009; Nisalak *et al.* 2003; Vaughn *et al.* 2000; Gubler, 1998]. The progression and duration of disease is usually resolved within 2 weeks after the initial inoculation of DENV by the mosquito vector (Figure 1). Subsequent to the bite of the insect, an incubation period (ranging from 3 to 7 days) occurs prior to the emergence of clinical symptoms, such as fever. However, infected patients do not generally seek professional help at the first sign of illness, but often wait until after an extensive febrile period (2–3 days) has passed. Thus, frequently, the disease conditions have worsened to the point requiring imminent attention and hospitalization for supportive or palliative care. The blood samples that are collected upon enrollment are obtained during the febrile stage of illness and usually at the peak of viremia. In general, patients undergoing the febrile and viremic stages do not experience severe symptoms. Subjects more often suffer from plasma leakage and/or shock at the time of viral clearance when the body temperature begins to return to normal, usually occurring 6–8 days after the initiation of fever. Dengue patients have registered dynamic spectrums of clinical manifestations [Gibbons and Vaughn, 2002; Gubler, 1998], which include typical fever (dengue fever [DF]), undifferentiated fever, and dengue hemorrhagic fever (DHF) with or without dengue shock syndrome (DSS). Although a majority of subjects

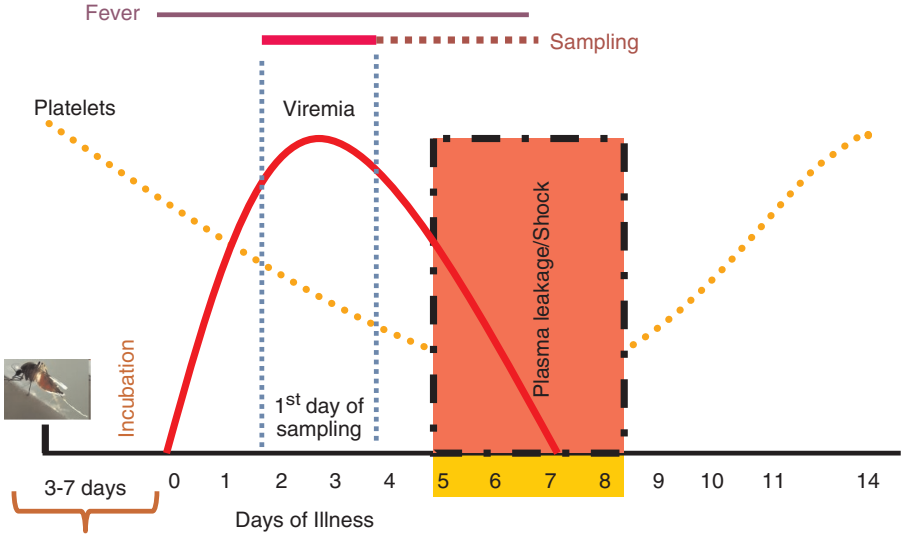

Figure 1. Chronology of events in dengue virus (DENV) infection. After the bite of the mosquito carrying infectious DENV, clinical symptoms, such as fever, appear after around 3–7 days of incubation. Viremia can be detected, likely at the onset of fever, and can last for 5–7 days. Patients normally do not seek help until after 2–3 days of fever; this is the time when the first blood sample is taken and often corresponds with the peak of viremia. Severe dengue disease, shock and plasma leakage frequently occurs at the time of or right after viral clearance and during the abatement of fever. The nadir in platelet counts are normally observed at the time of shock/plasma leakage, 5–8 days after fever illness.

experience DF, a self-limiting illness, some may progress to a much more severe and potential life-threatening form, DHF/DSS, characterized by increased vascular permeability, plasma leakage, and shock [WHO, 2009]. The factors contributing to DHF/DSS remain elusive in spite of many decades of intensive investigation. Several hypotheses on the potential risk factors have been suggested; to name a few: nutritional status, host genetic background, virus strain, and pre-existing immunity [Stephens, 2010; Kalayanarooj and Nimmannitya, 2005; Gubler, 1998; Halstead, 1988; Winter *et al.* 1968]. Although dengue may infect people from a wide range of ages, school-aged children appear to be the most vulnerable group (Figure 2) for ill-defined reasons. The death rate for patients experiencing DHF/DSS is around 2–5%, mainly in children under 15 years of age in some endemic countries [WHO, 2001].

Peripheral blood

Blood is the easiest to obtain and readily accessible. Consequently, thorough hematological profiling of this constituent from dengue patients has been well documented [Srichaikul and Nimmannitya, 2000; Halstead, 1982; Nelson *et al.* 1966]. The participation from the blood's many individual components, for instance the activation of complement [Suvatte, 1987; Bokisch *et al.* 1973], the status of monocytes and T-lymphocytes [Kurane, 1997; Kurane *et al.* 1990], and the levels of platelets [Mitrakul, 1987; Nimmannitya, 1987], can be a good prognostic tool to evaluate the stage of disease and severity of conditions. Several key findings from these investigations have been validated, such as the presence of pan-leucopenia and thrombocytopenia (Figure 3, from unpublished results), cells with an abnormal morphology (atypical lymphocytes) [Thisyakorn *et al.* 1984], and an altered ratio of immune cells [Chen *et al.* 2005]. Although some blood parameters have been investigated in great detail, the avenues leading to the development of many of these hematological changes remain to be resolved. For instance, the cause of thrombocytopenia in dengue patients has not been elucidated; its occurrence could be due to increased consumption, decreased production, or direct engagement of virus with platelets [Honda *et al.* 2009; Noisakran *et al.* 2009; WHO, 2009; Wang *et al.* 1995]. In addition, alterations of the pathophysiological parameters in dengue patients have been well investigated and reported [WHO, 1973].

Bone marrow

Owing to the intense pain patients feel, dengue has been referred to as breakbone fever, implying

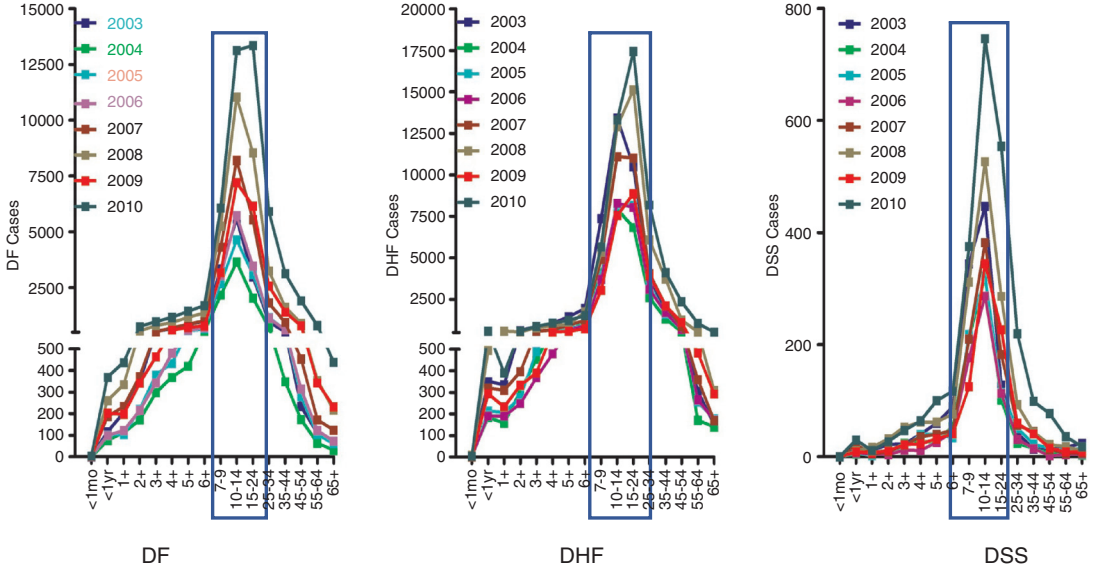

Figure 2. Age distribution and dengue morbidity. A wide spectrum of dengue diseases has be registered: dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The peak in morbidity is often observed with the 7- to 18-year-old cohorts in dengue endemic countries, such as Thailand. (*Source*: http://epid.moph.go.th).

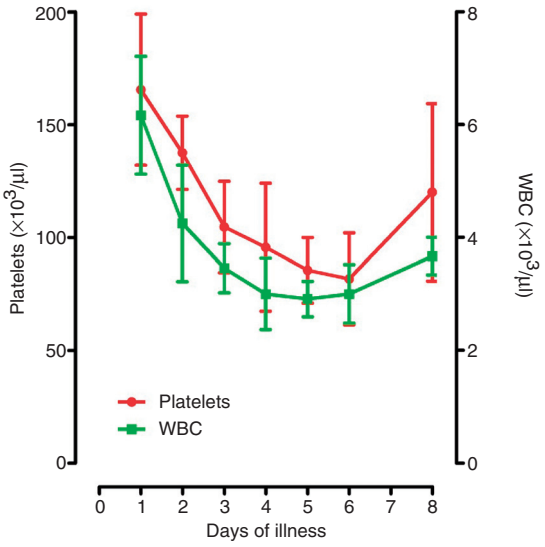

Figure 3. Thrombocytopenia and pancytopenia in dengue patients. Daily platelet and white blood cell counts are tabulated from dengue patients. The lowest levels always occur at the time of severe disease, 5–8 days after the fever.

that the bone marrow (BM) may somehow be involved in dengue pathogenesis. One early investigation on the cellularity of BM revealed that early BM suppression in dengue patients is a common phenomenon [Bierman and Nelson, 1965]. In addition, BM-associated aplasia in dengue patients has been documented, although infrequently [Srichaikul *et al.* 2008; Au *et al.* 2001; Young, 1990]. However, its involvement in DENV infection has not been well studied due to the difficulty of acquisition and potential bleeding risk to dengue patients. Consequently, the status of the BM involvement in dengue pathogenesis and the mechanisms leading to its suppression in patients has not been examined thoroughly. Nonetheless, available evidence suggests that the BM is involved at early stages of infection [La Russa and Innis, 1995; Nelson *et al.* 1966]. BM taken at the onset of fever registers abnormal BM cellularity (reduction in the levels of megakaryopoiesis, erythropoiesis and granulocytosis) and hypercellularity or normocellularity while taken at the time of severe clinical manifestations [Bhamarapravati *et al.* 1967, 1966; Aikat, 1966; Na-Nakorn *et al.* 1966; WHO, 1966; Piyaratn, 1961]. An unusual incidence of phagocytic reticulum cells has been revealed in BM taken at late stages of infection [Kho *et al.* 1972; Aikat, 1966; Na-Nakorn *et al.* 1966]. Importantly, these cells are overreactive and phagocytose all blood elements [Kho *et al.* 1972; Aikat, 1966]. Interestingly, hemaphagocytosis and lymphohistiocytosis in dengue patients has been registered, although infrequently [Jain and Singh, 2008; Lu *et al.* 2005; Chen *et al.* 2004; Rueda *et al.* 2002; Bhamarapravati *et al.* 1967; Na-Nakorn *et al.* 1966], and the mortality associated with this syndrome is very high [Sullivan, 2003].

Complications

With dynamic clinical manifestations, complications are a frequent event in dengue patients. The most common are acute renal failure, bacteremia, hepatitis, pleural effusion, and gastrointestinal bleeding [Tsai *et al.* 2011; Lee *et al.* 2009; WHO, 2009]. Complications associated with acute DENV infections have been recorded but the significance of these occurrences has not been systematically investigated. Also, a consensus has not been reached on whether individuals presenting with more amenable symptoms early in disease are more prone to severe dengue [WHO, 2009]. In general, during the early stage of infection, prior to the onset of DF or severe DHF/DSS, viremia is one of the key features observed in the circulation of patients, but it seldom leads to their death [WHO, 2009]. The critical period of disease often occurs at the end of viral clearance [Bethell *et al.* 2001; Srichaikul and Nimmannitya, 2000; Nimmannitya, 1987], presumably the time at which functional immune cells from the BM are restored. Consequently, an overabundance of immune system factors

can be expressed. This in conjunction with sudden changes in the pathophysiology of the microenvironment may result in a chain reaction of responses leading to the induction of complications, eventually accounting for the death of the patients.

Bench and applied research on disease pathogenesis

There are several ways to investigate phenomena in life sciences. Bench research and applied research or the so-called translational medicine research are the two most common varieties of studies that have been performed and frequently cited, however these two terms have been used somewhat interchangeably. The former often refers to experiments performed under ideal conditions, using model systems, to work out the biological details of a given molecule or pathogen. While the latter deals with a much more complicated system, often involving humans or patient samples for the purpose of evaluating the treatment of diseases. The most frequently implemented investigations performed today are of the bench research variety. However, translational medicine research is gaining the attention of investigators and gradually increasing in number. Virus–host interactions are a critical category to be evaluated for the evolution of disease. Dynamic layers of regulatory events exist, capable of defending the host, and each one of them has its own unique function in interacting with and balancing out the overall immune and physiological responses to the infection. Cumulative data reveals that there is a big difference between the information obtained from translational medicine type research and bench investigations. Therefore, the particular aspects of the research performed must be completely specified to mitigate the risk of misinforming the scientific community as to the relevance of the study results.

Bench research on dengue virus infection

Owing to the complexity in systemic infections, it is difficult to single out one key and critical parameter responsible for the outcome of a disease. Consequently, numerous factors, dependent upon the individual's interest, are opted out by investigators to simplify the explanation of the disease phenomenon. Among all of the cellular components in dengue patients, mononuclear phagocytic cells, monocytes/macrophages and dendritic cells are by far the most frequently investigated blood elements [Marovich *et al.* 2001; Palucka, 2000; Halstead *et al.* 1977]. The overemphasis of these cells' contribution to disease may be related to their potential role in enhancing DENV infection in an antibody-dependent fashion, or the alleged antibody-dependent enhancement (ADE) concept [Halstead, 1988; Halstead and O'Rourke, 1977]. This hypothesis proclaims that higher viremia and more severe dengue disease occurs in patients with secondary infections, however the only populations investigated are those inhabiting dengue endemic zones, where the majority of the population are seropositive by the age they enter elementary school [Sangkawibha *et al.* 1984]. In addition, an ill-defined mononuclear phagocytic cells subpopulation are suggested to be permissive to DENV, with the infectivity enhanced by the assistance of subneutralizing antibody [Beltramello *et al.* 2010; Dejnirattisai *et al.* 2010; Halstead *et al.* 1977]. However, the physical evidence is hard to prove *in vivo*. Interestingly, direct physical evidence, such as the presence of dengue viral particles in these cells, has not been provided so far. Instead, available evidence from *in vivo* investigations has demonstrated that dengue viral antigen in phagocytic cells is always seen at the end of or right after viral clearance [Marchette *et al.* 1973]. Since thrombocytopenia is a key clinical finding in dengue patients during the course of viremia, the involvement of platelets in DENV infection has been intensively investigated as well [Honda *et al.* 2009; Noisakran *et al.* 2009; Wang *et al.* 1995; Boonpucknavig *et al.* 1979; Scott, 1978]. Evidence includes the deposition of dengue viral antigen and the appearance of viral particles inside of platelets isolated from patients. This suggests that DENV may circulate under the shield of the platelet plasma membrane [Noisakran *et al.* 2009; Boonpucknavig *et al.* 1979]. Perhaps, this may be the reason platelet-associated immunoglobulin M (PAIgM) or G (PAIgG) has been observed [Noisakran and Perng, 2008; Saito *et al.* 2004; Oishi *et al.* 2003]. Thrombocytopenia may be the result of the interactions of platelets containing viral particles and PAIgM/PAIgG [Honda *et al.* 2009; Oishi *et al.* 2007]. In addition, engulfment of dengue viral infected platelets by monocytes is observed in blood smears from infected nonhuman primates [Onlamoon *et al.* 2010] and the importance of monocytes/macrophages in the control of DENV infection has been emphasized [Fink *et al.* 2009]. Recently, flow cytometric analyses with hospitalized dengue patients depict that platelet–leukocyte aggregates, especially platelet–

monocyte aggregates, are commonplace during the viral clearance stage [Tsai *et al.* 2011]. Furthermore, these results have been confirmed with blood smears prepared from acute dengue patients, revealing that virus infected platelets are engulfed by monocytes (Figure 4, from unpublished results). This evidence suggests that the main role of these phagocytic cells is to engulf crippled and damaged cells during the course of DENV infection. However, whether subneutralizing antibody can enhance phagocytosis remains to be further explored *in vivo*.

Although much knowledge and information has been generated from these phagocytic cells with DENV *in vitro*, the clinical relevance has not been well established. There are several possible factors that may explain the differences. First, literature searches reveal that DENV infected monocytes/macrophages or dendritic cells are hardly seen in dengue patients in spite of high viremia [WHO, 2009]. Second, although *in vitro* experiments are capable of illustrating the occurrence of ADE, physical appearance of dengue viral particles inside these phagocytic cells has not been demonstrated. In contrast, apoptosis of these cells upon infection by DENV is a constant finding [Torrentes-Carvalho *et al.* 2009; Mosquera *et al.* 2005; Navarro-Sanchez *et al.* 2005]. Third, even when these cells are inoculated in the presence of antibodies, only a low percentage of cells become infected. Also the DENV-positive populations from these experiments are never clearly defined with cell surface markers [Boonnak *et al.* 2011]. Lastly, in nonendemic zones, primary or naïve individuals can succumb to severe dengue symptoms [Meltzer and Schwartz, 2009], suggesting an alternate mechanism may play a role in dengue pathogenesis. Host factors have been suggested as an explanation for the occurrence of disease in DENV infections [Stephens, 2010]. Although some clinical features can be seen with different animal models [Noisakran *et al.* 2010; Yauch and Shresta, 2008; Bente and Rico-Hesse, 2006], the reality is that the lack of a suitable model that can recapitulate the cardinal features of dengue disease observed in humans has vastly hindered progress. The most important mechanisms leading to severe

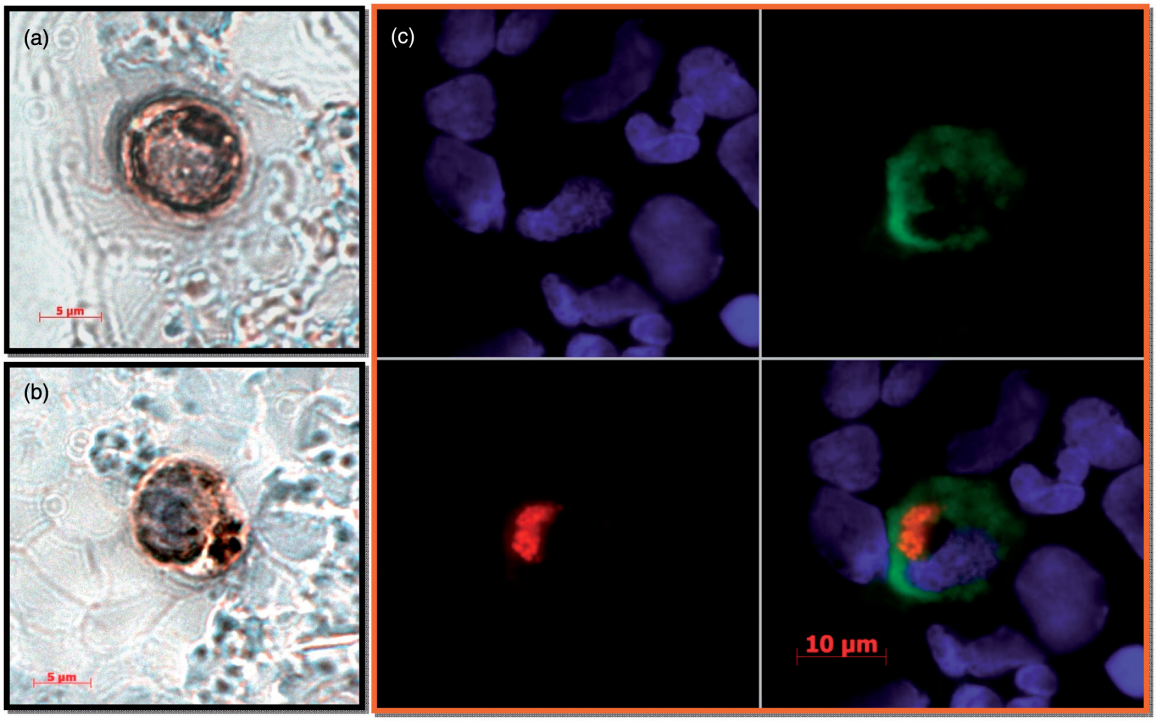

Figure 4. Engulfment of dengue-infected platelets by monocytes. Immunohistochemical stainings were performed with blood smears from acute dengue patients. (A) Smear stained with dengue-specific monoclonal antibody (clone 3H5). (B) Smear stained with platelet-specific marker, CD61. (C) Smear stained with monocyte specific marker, CD14 (green), and platelet specific marker, CD41a (red).

manifestations of dengue disease *in vivo* remain an enigma.

Applied research on dengue pathogenesis

Understanding pathogenesis is probably the most important aspect, giving clues to designing accurate treatments to ameliorate disease. As mentioned previously, dengue patients generally do not seek professional help until the late stage of fever, often after 2–3 days of clinical illness (Figure 5, from unpublished results). Samples collected at the time of the patient's visit are always quite late although they are often at the peak of DF symptoms.

Parameters obtained from these samples after analysis could be interpreted in many ways. Often, the terms *association* or *correlation* are used to identify a likely link between the tested variable and the disease condition as well as to ascribe a possible mechanism explaining the progression of disease. For instance, many researchers have investigated the frequencies of alleles present in DF/DHF/DSS patients and implicated specific host factors to be likely *associated* and consequent factors playing a major role in dengue pathogenesis [Stephens, 2010]. While some of these factors were initially suggested to be causative for severe dengue in animal models, usually systematic investigations reveal that these factors are not capable of predicting the severity of symptoms in humans. For example, a recent report suggests that CLEC5A is critical for DENV-induced lethal disease in a mouse model [Chen *et al.* 2008]. This protein is a member of the C-type lectin superfamily and, in humans, it is encoded by the *CLEC5A* gene containing 7 exons that are spliced together to form the complete CLEC5A RNA and protein. It is one of many proteins that are expressed on the surface of peripheral blood monocytes and has not been assigned a specific function. Considering the report by Chen and colleagues on the importance of monocytes and CLEC5A in DENV infection, it was appropriate to conduct a retrospective study with the aim to associate *CLEC5A* alleles with the risk of acquiring severe dengue. An Institutional Review Board (IRB) approved study was conducted, involving the careful screening and enrollment of 100 confirmed pre-exposed and recovered dengue patients. The demography of the enrollees were: 49 with DF (male:female, 22:27; mean age 54 (7–78)), 51 with DHF (male:female, 30:21; mean age 53 (7–78)). However, our systematic investigation with a well-designed retrospective cohort study revealed that the role of CLEC5A in dengue pathogenesis seems to be insignificant (Table 1).

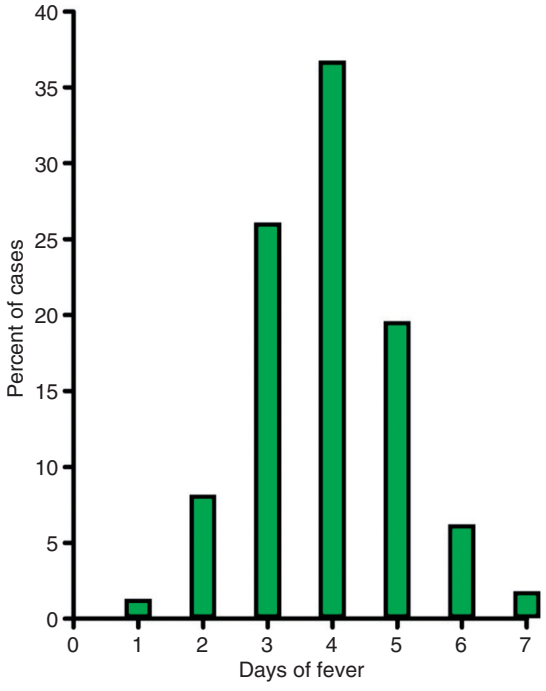

Figure 5. Dengue patients seek professional help after fever. Cumulative data indicates that the majority of dengue patients arrive at hospitals or clinics after 2–3 days of fever.

Therefore, this protein is not likely to play a significant role in dengue pathogenesis. Thus, the animal model may be suitable to investigate some aspects of disease conditions but often does not translate into useful information that is applicable to humans. This observation also indicates that an alternative mechanism may remain to be explored.

Current proposed concept

Differences in clinical features with the age of patient have been documented. Young children often present with an undifferentiated febrile illness with maculopapular rash [Burke *et al.* 1988]. In contrast, older children, adolescents, and adults more commonly display with classic dengue [Sharp *et al.* 1995]. Furthermore DHF has been recognized as a disease primarily occurring in children under 15 years in hyperendemic areas, but in other regions, adults appear to be more likely to develop with severe manifestations [Guilarde *et al.* 2008; Guzman and Kouri, 2003]. The reasons for the geographical difference in

Table 1. Analysis of single nucleotide polymorphisms (SNPs) of CLEC5A in dengue patients. Seven exons encoding CLEC5A are amplified by polymerase chain reaction (PCR). The nucleotides of exon1 to exon7 of the CLEC5A and their flanking sequences with introns were verified by resequencing the genomic DNA. Any SNPs identified from the above 'resequencing' procedure were selected for genotyping on all patients.

rs-ID[a]	chr_NO	Position	Gene name	*p*-value (genotype) DF *versus* DHF[b]	*p*-value (allele) DF *versus* DHF[b]
rs11770855	chr7	141275173	CLEC5A	0.724315511	0.519362523
rs12668558	chr7	141280116	CLEC5A	0.390447284	0.386847686
rs12673916	chr7	141278039	CLEC5A	No polymorphism	No polymorphism
rs1285933	chr7	141273618	CLEC5A	0.210614242	0.132124504
rs1285935	chr7	141274408	CLEC5A	0.693786259	0.381762789
rs1285936	chr7	141277305	CLEC5A	No polymorphism	No polymorphism
rs1285945	chr7	141286110	CLEC5A	0.359163485	0.50216537
rs1285946	chr7	141286058	CLEC5A	0.884244702	0.836872151
rs1285947	chr7	141286014	CLEC5A	0.361760277	0.532550163
rs1285952	chr7	141282649	CLEC5A	No polymorphism	No polymorphism
rs1285968	chr7	141293491	CLEC5A	0.70944803	0.546210762
rs1294352	chr7	141281370	CLEC5A	0.359163485	0.50216537
rs17162587	chr7	141292242	CLEC5A	0.145800006	0.449577069
rs17634147	chr7	141277561	CLEC5A	No polymorphism	No polymorphism
rs2204608	chr7	141278821	CLEC5A	No polymorphism	No polymorphism
rs2293459	chr7	141291709	CLEC5A	0.359163485	0.50216537
rs2293460	chr7	141292899	CLEC5A	0.965059042	0.79195191
rs2293461	chr7	141292903	CLEC5A	0.972088073	0.822674085
rs35942193	chr7	141278019	CLEC5A	No polymorphism	No polymorphism
rs6943066	chr7	141281146	CLEC5A	No polymorphism	No polymorphism
rs6960597	chr7	141281447	CLEC5A	No polymorphism	No polymorphism

[a]SNP rs-ID is retrieved from SNP Home, http://www.ncbi.nlm.nih.gov/snp.
[b]Statistical analyses are performed with SPSS13.0, $p < 0.05$ is considered significant.
DF, dengue fever; DHF, dengue hemorrhagic fever.

disease severity are currently unknown. Some advocate different viral strains in circulation [Guilarde *et al.* 2008; Messer *et al.* 2003], while others assume the ethic genetic background differences can explain this [Halstead *et al.* 2001]. Nevertheless, as a whole, DENV can infect people of a wide range of ages; severe disease is particularly common in school-aged children in South-East Asia for reasons that remain poorly understood. However, one possibility for the differences in disease presentation relates to the cellular composition of the BM, which is known to change with age [Bain *et al.* 2010; Glaser *et al.* 1950]. Hence, an alternate hypothesis explaining the susceptibility of individuals to severe dengue may reside in the BM compartment.

BM accounts for approximately 5% of the body weight in humans [Picker, 1999] and is the major hematopoietic organ. It consists of hematopoietic tissue islands and vascular sinuses surrounded by adipose cells and interspersed within a meshwork of trabecular bone [Travlos, 2006]. The composition of the two proportions, red (hematopoietically active) and yellow adipose tissue (inactive), in the BM vary at any given time, dependent on the age and health of the person [Bain *et al.* 2010; Litwiejko-Pietrynczak *et al.* 2004; Compston, 2002; Morrison *et al.* 1996; Glaser *et al.* 1950]. Although at birth red marrow is dominant, it converts from mainly hematopoietic to more white fatty cells as the child grows older. Around the age of 5, the red marrow begins to be gradually replaced by yellow, with the process completed by adulthood [Bain *et al.* 2010; Litwiejko-Pietrynczak *et al.* 2004]. However, this process is somewhat reversible; the yellow bone marrow has the ability to convert back into red marrow within a couple of hours, in the case of large volume blood losses. Although DENV infects a wide range of age groups, the peak of DHF appears to be within the school-aged cohort (Figure 2). Thus, it is conceivable that the differential composition of the BM could be playing a significant role in dengue pathogenesis.

Interestingly, there are some reports on the involvement of BM in other families of viral hemorrhagic fever viruses, suggesting that to some

extent abnormal vascular regulation and vessel damage caused by members of the Arenaviviridae, Filoviridae, Bunyaviridae, and Flaviviridae families of viruses might share a common pathogenetic mechanism. Morphological investigations of the postmortem biopsies in Ebola virus (family Filoviridae) infected BM implicated that a direct effect of virus on granulocyte precursors is likely [Dietrich, 1977]. Hemophagocytosis has been observed in Crimean–Congo hemorrhagic fever (CCHF) induced by CCHF virus (family Bunyaviridae) infection [Karti *et al.* 2004]. As mentioned earlier, hemophagocytosis can be observed in dengue patients in the phase of BM recovery as well [Jain and Singh, 2008; Lu *et al.* 2005; Chen *et al.* 2004; Rueda *et al.* 2002; Bhamarapravati *et al.* 1967; Na-Nakorn *et al.* 1966]. However, in Lassa virus hemorrhagic fever induced by Lassa virus (family Arenaviridae), evidence accumulative thus far suggests that a different pathogenetic mechanism is likely involved since leukocyte and platelet counts are often normal in these infected patients [Knobloch *et al.* 1980]. In short, the fine underlying mechanisms leading to the vascular damage in various viral hemorrhagic fevers caused by various viruses remain to be further elucidated.

As mentioned above, BM is one of the target organs involved in dengue pathogenesis, as evidenced by the presence of hypoplasia. BM suppression, in particular the reduction of cellularity at early time points of infection in dengue patients, is a well-known and documented fact. Importantly, virus acquired by the transfusion of BM from an infected donor has indicated that its involvement must be very early, prior to the development of clinical symptoms [Rigau-Perez *et al.* 2001]. In addition, it has been demonstrated with biopsy results from dengue patients at the early phase of disease that the BM cells, especially those of the megakaryocyte lineage, have been diminished [Aikat, 1966; Bhamarapravati *et al.* 1966; Nelson *et al.* 1966]. Also dengue-infected patient BM more frequently yields virus in culture than the peripheral blood [Bierman and Nelson, 1965]. Furthermore, *ex vivo* experimental studies have revealed that DENV can efficiently infect hematopoietic cells [Bente *et al.* 2005; Nakao *et al.* 1989] and is only capable of replication in leukocytes derived from BM and not from other lymphatic tissues (spleen, thymus and lymph node) [Halstead *et al.* 1977]. Evidence cumulated thus far points toward the hematopoietic progenitor cells playing a very significant role in dengue pathogenesis. Whether the fluctuations in the cellular composition of the BM, which occurs as a function of age, can account for the differences in susceptibility to DENV warrants further investigation. Furthermore, suppression of the megakaryocytes in dengue patients has been noted but the significance of the phenomenon to clinical disease requires more attention in order to better define the mechanisms leading to severe dengue disease.

Conclusion and summary

Intensive efforts have been made to comprehend the pathogenesis of DENV infection utilizing data obtained from studies using peripheral blood components from patients, *in vitro* cell culture and imperfect animal models. Despite all of these efforts, the actual causative factors accounting for severe disease remain an enigma. Indeed, interpretation of the data obtained from these systems should be cautiously considered and accurately reported, without distorting its relevance to clinical settings. New avenues must be explored to ascertain how disease progresses in humans. New topics to be investigated include but are not limited to the direct involvement of the BM progenitor cells in dengue pathogenesis. These include deciphering whether the hematopoietic cells can augment bleeding and plasma leakage, and determining whether the BM can sustain virus infection and contribute to the viremia observed in the peripheral blood of the patients. In addition, cytokine studies describing the alterations in progenitor cell potential or cellular differentiation are lacking in DENV-infected BM. This information is needed for a better and perhaps more complete understanding of the pathogenesis and involvement of the BM in DENV infection. Accordingly, this review has emphasized the importance of understanding the role of the BM by highlighting the appropriate studies. Knowledge pertaining to the virus interactions with the progenitor cells of the bone marrow will provide new insight for vaccine development and therapeutic drug design and will pave the way for better patient-oriented care.

Authors' contributions

JJT initiated the concept, designed the study, wrote and obtained the IRB protocol, and participated in drafting the manuscript. LTL carried out the molecular genetics on CLEC5A studies

and participated in the sequence alignment. KC assisted in the enrollment of the patients and drafted the manuscript. SHW participated in molecular genetic studies and in the sequence alignment, and performed the statistical analysis. HMH carried out the immunostaining and assays. KBC participated in the design of the study, drafted and edited the manuscript. GCP participated in study design and coordination, and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgement

We would like to thank the clinical staff at the Tropical Medicine Center and Division of Infectious Diseases of Kaohsiung Medical University Hospital.

Funding

This study was supported by Emory SOM start-up fund and Grants from Taiwan Center for Disease Control and Taiwan National Science Council.

Conflict of interest statement

The authors declare that they have no conflict of interests.

References

Aikat, B.K. (1966) Pathology of mosquito-borne haemorrhagic fever in the Calcutta outbreak. *Bull World Health Organ* 35: 48–49.

Au, W.Y., Ma, E.S. and Kwong, Y.L. (2001) Acute myeloid leukemia precipitated by dengue virus infection in a patient with hemoglobin H disease. *Haematologica* 86: E17.

Bain, B.J., Clark, D.M. and Wilkins, B.S. (2010) Bone Marrow Pathology, 4th edn, Hoboken, NJ: Wiley-Blackwell.

Beltramello, M., Williams, K.L., Simmons, C.P., Macagno, A., Simonelli, L., Quyen, N.T. *et al.* (2010) The human immune response to Dengue virus is dominated by highly cross-reactive antibodies endowed with neutralizing and enhancing activity. *Cell Host Microbe* 8: 271–283.

Bente, D.A., Melkus, M.W., Garcia, J.V. and Rico-Hesse, R. (2005) Dengue fever in humanized NOD/SCID mice. *J Virol* 79: 13797–13799.

Bente, D.A. and Rico-Hesse, R. (2006) Models of dengue virus infection. *Drug Discov Today Dis Models* 3: 97–103.

Bethell, D.B., Gamble, J., Pham, P.L., Nguyen, M.D., Tran, T.H., Ha, T.H. *et al.* (2001) Noninvasive measurement of microvascular leakage in patients with dengue hemorrhagic fever. *Clin Infect Dis* 32: 243–253.

Bhamarapravati, N., Boonyapaknavik, V. and Nimsomburana, P. (1966) Pathology of Thai haemorrhagic fever: an autopsy study. *Bull World Health Organ* 35: 47–48.

Bhamarapravati, N., Tuchinda, P. and Boonyapaknavik, V. (1967) Pathology of Thailand haemorrhagic fever: a study of 100 autopsy cases. *Ann Trop Med Parasitol* 61: 500–510.

Bierman, H.R. and Nelson, E.R. (1965) Hematodepressive virus diseases of Thailand. *Ann Intern Med* 62: 867–884.

Bokisch, V.A., Top Jr, F.H., Russell, P.K., Dixon, F.J. and Muller-Eberhard, H.J. (1973) The potential pathogenic role of complement in dengue hemorrhagic shock syndrome. *N Engl J Med* 289: 996–1000.

Boonnak, K., Dambach, K.M., Donofrio, G.C., Tassaneetrithep, B. and Marovich, M.A. (2011) Cell type specificity and host genetic polymorphisms influence antibody-dependent enhancement of dengue virus infection. *J Virol* 85: 1671–1683.

Boonpucknavig, S., Vuttiviroj, O., Bunnag, C., Bhamarapravati, N. and Nimmanitya, S. (1979) Demonstration of dengue antibody complexes on the surface of platelets from patients with dengue hemorrhagic fever. *Am J Trop Med Hyg* 28: 881–884.

Burke, D.S., Nisalak, A., Johnson, D.E. and Scott, R.M. (1988) A prospective study of dengue infections in Bangkok. *Am J Trop Med Hyg* 38: 172–180.

Chen, C.J., Huang, Y.C., Jaing, T.H., Hung, I.J., Yang, C.P., Chang, L.Y. *et al.* (2004) Hemophagocytic syndrome: a review of 18 pediatric cases. *J Microbiol Immunol Infect* 37: 157–163.

Chen, R.F., Liu, J.W., Yeh, W.T., Wang, L., Chang, J.C., Yu, H.R. *et al.* (2005) Altered T helper 1 reaction but not increase of virus load in patients with dengue hemorrhagic fever. *FEMS Immunol Med Microbiol* 44: 43–50.

Chen, S.T., Lin, Y.L., Huang, M.T., Wu, M.F., Cheng, S.C., Lei, H.Y. *et al.* (2008) CLEC5A is critical for dengue-virus-induced lethal disease. *Nature* 453: 672–676.

Compston, J.E. (2002) Bone marrow and bone: a functional unit. *J Endocrinol* 173: 387–394.

Dejnirattisai, W., Jumnainsong, A., Onsirisakul, N., Fitton, P., Vasanawathana, S., Limpitikul, W. *et al.* (2010) Cross-reacting antibodies enhance dengue virus infection in humans. *Science* 328: 745–748.

Dietrich, M., Schumacher, H.H., Peters, D. and Knobloch, J. (1977) Human pathology of ebola (Maridi) virus infection in the Sudan. In: *Proceedings of an International Colloquium on Ebola Virus Infection and Other Haemorrhagic Fevers, Antwerp, Belgium*, Amsterdam: Elsevier/North-Holland Biomedical Press.

Fink, J., Gu, F. and Vasudevan, S.G. (2006) Role of T cells, cytokines and antibody in dengue fever and dengue haemorrhagic fever. *Rev Med Virol* 16: 263–275.

Fink, K., Ng, C., Nkenfou, C., Vasudevan, S.G., van Rooijen, N. and Schul, W. (2009) Depletion of macrophages in mice results in higher dengue virus titers and highlights the role of macrophages for virus control. *Eur J Immunol* 39: 2809–2821.

Gibbons, R.V. and Vaughn, D.W. (2002) Dengue: an escalating problem. *BMJ* 324: 1563–1566.

Glaser, K., Limarzi, L.R. and Poncher, H.G. (1950) Cellular composition of the bone marrow in normal infants and children. *Pediatrics* 6: 789–824.

Gubler, D.J. (1998) Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 11: 480–496.

Gubler, D.J., Suharyono, W., Tan, R., Abidin, M. and Sie, A. (1981) Viraemia in patients with naturally acquired dengue infection. *Bull World Health Organ* 59: 623–630.

Guilarde, A.O., Turchi, M.D., Siqueira Jr, J.B., Feres, V.C., Rocha, B., Levi, J.E. *et al.* (2008) Dengue and dengue hemorrhagic fever among adults: clinical outcomes related to viremia, serotypes, and antibody response. *J Infect Dis* 197: 817–824.

Guzman, M.G., Halstead, S.B., Artsob, H., Buchy, P., Farrar, J., Gubler, D.J. *et al.* (2010) Dengue: a continuing global threat. *Nat Rev Microbiol* 8(12 Suppl): S7–S16.

Guzman, M.G. and Kouri, G. (2003) Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. *J Clin Virol* 27: 1–13.

Halstead, S.B. (1982) Dengue: hematologic aspects. *Semin Hematol* 19: 116–131.

Halstead, S.B. (1988) Pathogenesis of dengue: challenges to molecular biology. *Science* 239: 476–481.

Halstead, S.B. (2007) Dengue. *Lancet* 370: 1644–1652.

Halstead, S.B. and O'Rourke, E.J. (1977) Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med* 146: 201–217.

Halstead, S.B., O'Rourke, E.J. and Allison, A.C. (1977) Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection. *J Exp Med* 146: 218–229.

Halstead, S.B., Streit, T.G., Lafontant, J.G., Putvatana, R., Russell, K., Sun, W. *et al.* (2001) Haiti: absence of dengue hemorrhagic fever despite hyperendemic dengue virus transmission. *Am J Trop Med Hyg* 65: 180–183.

Honda, S., Saito, M., Dimaano, E.M., Morales, P.A., Alonzo, M.T., Suarez, L.A. *et al.* (2009) Increased phagocytosis of platelets from patients with secondary Dengue virus infection by human macrophages. *Am J Trop Med Hyg* 80: 841–845.

Jain, D. and Singh, T. (2008) Dengue virus related hemophagocytosis: a rare case report. *Hematology* 13: 286–288.

Kalayanarooj, S. and Nimmannitya, S. (2005) Is dengue severity related to nutritional status?. *Southeast Asian J Trop Med Public Health* 36: 378–384.

Karti, S.S., Odabasi, Z., Korten, V., Yilmaz, M., Sonmez, M., Caylan, R. *et al.* (2004) Crimean-Congo hemorrhagic fever in Turkey. *Emerg Infect Dis* 10: 1379–1384.

Kho, L.K., Wulur, H. and Himawan, T. (1972) Blood and bone marrow changes in dengue haemorrhagic fever. *Paediatr Indones* 12: 31–39.

Knobloch, J., McCormick, J.B., Webb, P.A., Dietrich, M., Schumacher, H.H. and Dennis, E. (1980) Clinical observations in 42 patients with Lassa fever. *Tropenmed Parasitol* 31: 389–398.

Kurane, I. and Ennis, F.A. (1997) Immunopathogenesis of dengue virus infections, In: Gubler, D.J. and Kuno, G. (eds). Dengue and Dengue Hemorrhagic Fever, Wallingford, UK: CAB International, pp. 273–290.

Kurane, I., Innis, B.L., Nimmannitya, S., Nisalak, A., Rothman, A.L., Livingston, P.G. *et al.* (1990) Human immune responses to dengue viruses. *Southeast Asian J Trop Med Public Health* 21: 658–662.

La Russa, V.F. and Innis, B.L. (1995) Mechanisms of dengue virus-induced bone marrow suppression. *Baillieres Clin Haematol* 8: 249–270.

Lee, I.K., Liu, J.W. and Yang, K.D. (2009) Clinical characteristics, risk factors, and outcomes in adults experiencing dengue hemorrhagic fever complicated with acute renal failure. *Am J Trop Med Hyg* 80: 651–655.

Litwiejko-Pietrynczak, E., Szkudlarek, M., Klim, B. and Pietrewicz, T.M. (2004) Bone marrow megakaryocytes in human ontogenesis. *Rocz Akad Med Bialymst* 49(Suppl. 1): 210–212.

Lu, P.L., Hsiao, H.H., Tsai, J.J., Chen, T.C., Feng, M.C., Chen, T.P. *et al.* (2005) Dengue virus-associated hemophagocytic syndrome and dyserythropoiesis: a case report. *Kaohsiung J Med Sci* 21: 34–39.

Marchette, N.J., Halstead, S.B., Falkler Jr, W.A., Stenhouse, A. and Nash, D. (1973) Studies on the pathogenesis of dengue infection in monkeys. 3. Sequential distribution of virus in primary and heterologous infections. *J Infect Dis* 128: 23–30.

Marovich, M., Grouard-Vogel, G., Louder, M., Eller, M., Sun, W., Wu, S.J. *et al.* (2001) Human dendritic cells as targets of dengue virus infection. *J Investig Dermatol Symp Proc* 6: 219–224.

Martina, B.E., Koraka, P. and Osterhaus, A.D. (2009) Dengue virus pathogenesis: an integrated view. *Clin Microbiol Rev* 22: 564–581.

Meltzer, E. and Schwartz, E. (2009) A travel medicine view of dengue and dengue hemorrhagic fever. *Travel Med Infect Dis* 7: 278–283.

Messer, W.B., Gubler, D.J., Harris, E., Sivananthan, K. and de Silva, A.M. (2003) Emergence and global spread of a dengue serotype 3, subtype III virus. *Emerg Infect Dis* 9: 800–809.

Mitrakul, C. (1987) Bleeding problem in dengue haemorrhagic fever: platelets and coagulation changes. *Southeast Asian J Trop Med Public Health* 18: 407–412.

Morens, D.M. and Fauci, A.S. (2008) Dengue and hemorrhagic fever: a potential threat to public health in the United States. *JAMA* 299: 214–216.

Morrison, S.J., Wandycz, A.M., Akashi, K., Globerson, A. and Weissman, I.L. (1996) The aging of hematopoietic stem cells. *Nat Med* 2: 1011–1016.

Mosquera, J.A., Hernandez, J.P., Valero, N., Espina, L.M. and Anez, G.J. (2005) Ultrastructural studies on dengue virus type 2 infection of cultured human monocytes. *Virol J* 2: 26.

Murphy, B.R. and Whitehead, S.S. (2011) Immune response to dengue virus and prospects for a vaccine. *Annu Rev Immunol* 29: 587–619.

Na-Nakorn, S., Suingdumrong, A., Pootrakul, S. and Bhamarapravati, N. (1966) Bone-marrow studies in Thai haemorrhagic fever. *Bull World Health Organ* 35: 54–55.

Nakao, S., Lai, C.J. and Young, N.S. (1989) Dengue virus, a flavivirus, propagates in human bone marrow progenitors and hematopoietic cell lines. *Blood* 74: 1235–1240.

Navarro-Sanchez, E., Despres, P. and Cedillo-Barron, L. (2005) Innate immune responses to dengue virus. *Arch Med Res* 36: 425–435.

Nelson, E.R. and Bierman, H.R. (1964) Dengue fever: a thrombocytopenic disease?. *JAMA* 190: 99–103.

Nelson, E.R., Bierman, H.R. and Chulajata, R. (1966) Hematologic phagocytosis in postmortem bone marrows of dengue hemorrhagic fever. (Hematologic phagocytosis in Thai hemorrhagic fever). *Am J Med Sci* 252: 68–74.

Nelson, E.R., Tuchinda, S., Bierman, H.R. and Chulajata, R. (1966) Haematology of Thai haemorrhagic fever (dengue). *Bull World Health Organ* 35: 43–44.

Nimmannitya, S. (1987) Clinical spectrum and management of dengue haemorrhagic fever. *Southeast Asian J Trop Med Public Health* 18: 392–397.

Nisalak, A., Endy, T.P., Nimmannitya, S., Kalayanarooj, S., Thisayakorn, U., Scott, R.M. *et al.* (2003) Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. *Am J Trop Med Hyg* 68: 191–202.

Noisakran, S., Gibbons, R.V., Songprakhon, P., Jairungsri, A., Ajariyakhajorn, C., Nisalak, A. *et al.* (2009) Detection of dengue virus in platelets isolated from dengue patients. *Southeast Asian J Trop Med Public Health* 40: 253–262.

Noisakran, S., Onlamoon, N., Songprakhon, P., Hsiao, H.M., Chokephaibulkit, K. and Perng, G.C. (2010) Cells in dengue virus infection in vivo. *Adv Virol*, in press.

Noisakran, S. and Perng, G.C. (2008) Alternate hypothesis on the pathogenesis of dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) in dengue virus infection. *Exp Biol Med (Maywood)* 233: 401–408.

Oishi, K., Inoue, S., Cinco, M.T., Dimaano, E.M., Alera, M.T., Alfon, J.A. *et al.* (2003) Correlation between increased platelet–associated IgG and thrombocytopenia in secondary dengue virus infections. *J Med Virol* 71: 259–264.

Oishi, K., Saito, M., Mapua, C.A. and Natividad, F.F. (2007) Dengue illness: clinical features and pathogenesis. *J Infect Chemother* 13: 125–133.

Onlamoon, N., Noisakran, S., Hsiao, H.M., Duncan, A., Villinger, F., Ansari, A.A. *et al.* (2010) Dengue virus-induced hemorrhage in a nonhuman primate model. *Blood* 115: 1823–1834.

Palucka, A.K. (2000) Dengue virus and dendritic cells. *Nat Med* 6: 748–749.

Picker, L.J. and Siegelman, M.H. (1999) Lymphoid tissues and organs, In: Paul, W.E. (ed.). Fundamental Immunology, 4th edn, Philadelphia, PA: Lippincott-Raven, pp. 479–531.

Piyaratn, P. (1961) Pathology of Thailand epidemic hemorrhagic fever. *Am J Trop Med Hyg* 10: 767–772.

Rico-Hesse, R. (2009) Dengue virus markers of virulence and pathogenicity. *Future Virol* 4: 581.

Rigau-Perez, J.G., Vorndam, A.V. and Clark, G.G. (2001) The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994–1995. *Am J Trop Med Hyg* 64: 67–74.

Rueda, E., Mendez, A. and Gonzalez, G. (2002) [Hemophagocytic syndrome associated with dengue hemorrhagic fever]. *Biomedica* 22: 160–166.

Saito, M., Oishi, K., Inoue, S., Dimaano, E.M., Alera, M.T., Robles, A.M. *et al.* (2004) Association of increased platelet-associated immunoglobulins with thrombocytopenia and the severity of disease in secondary dengue virus infections. *Clin Exp Immunol* 138: 299–303.

Sangkawibha, N., Rojanasuphot, S., Ahandrik, S., Viriyapongse, S., Jatanasen, S., Salitul, V. *et al.* (1984) Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *Am J Epidemiol* 120 653–669.

Scott, R.M., Nisalak, A., Cheam-U-Dom, U., Seridhoranakul, S. and Nimmannitya, S. (1978) A preliminary report on the isolation of viruses from the

platelets and leukocytes of dengue patients. *Asian J Infectious Dis* 2: 95–97.

Sharp, T.W., Wallace, M.R., Hayes, C.G., Sanchez, J.L., DeFraites, R.F., Arthur, R.R. *et al.* (1995) Dengue fever in U.S. troops during Operation Restore Hope, Somalia, 1992–1993. *Am J Trop Med Hyg* 53: 89–94.

Srichaikul, T. and Nimmannitya, S. (2000) Haematology in dengue and dengue haemorrhagic fever. *Baillieres Best Pract Res Clin Haematol* 13: 261–276.

Srichaikul, T., Punyagupta, S., Kanchanapoom, T., Chanokovat, C., Likittanasombat, K. and Leelasiri, A. (2008) Hemophagocytic syndrome in Dengue hemorrhagic fever with severe multiorgan complications. *J Med Assoc Thai* 91: 104–109.

Stephens, H.A. (2010) HLA and other gene associations with dengue disease severity. *Curr Top Microbiol Immunol* 338: 99–114.

Sullivan, J.L. and Woda, B.A. (2003) Lymphohistiocytic disorders, In: David, G.N., Ginsburg, D., OrKin, S.H. and Look, A.T. (eds). Nathan and Oski's Hematology of Infancy and Chidhood, 6th edn, Philadelphia, PA: Saunders, pp. 1380–1381.

Suvatte, V. (1987) Immunological aspects of dengue haemorrhagic fever: studies in Thailand. *Southeast Asian J Trop Med Public Health* 18: 312–315.

Thisyakorn, U., Nimmannitya, S., Ningsanond, V. and Soogarun, S. (1984) Atypical lymphocyte in dengue hemorrhagic fever: its value in diagnosis. *Southeast Asian J Trop Med Public Health* 15: 32–36.

Torrentes-Carvalho, A., Azeredo, E.L., Reis, S.R., Miranda, A.S., Gandini, M., Barbosa, L.S. *et al.* (2009) Dengue-2 infection and the induction of apoptosis in human primary monocytes. *Mem Inst Oswaldo Cruz* 104: 1091–1099.

Travlos, G.S. (2006) Normal structure, function, and histology of the bone marrow. *Toxicol Pathol* 34: 548–565.

Tsai, J.J., Jen, Y.H., Chang, J.S., Hsiao, H.M., Noisakran, S. and Perng, G.C. (2011) Frequency alterations in key innate immune cell components in the peripheral blood of dengue patients detected by FACS analysis. *J Innate Immun*, in press.

Vaughn, D.W., Green, S., Kalayanarooj, S., Innis, B.L., Nimmannitya, S., Suntayakorn, S. *et al.* (2000) Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 181: 2–9.

Wang, S., He, R., Patarapotikul, J., Innis, B.L. and Anderson, R. (1995) Antibody-enhanced binding of dengue-2 virus to human platelets. *Virology* 213: 254–257.

WHO. (1966) Summaries of Papers Presented at the WHO Inter-Regional Seminar on Mosquito-borne Haemorrhagic Fevers in the South-East Asia and Western Pacific Regions. *Bull WHO* 35.

WHO. (1973) Pathogenetic mechanisms in dengue haemorrhagic fever: report of an international collaboratorive study. *Bull WHO* 48: 117–133.

WHO. (2009) Dengue and dengue hemorrhagic fever. *Bull WHO* 117.

WHO (2001) Dengue and dengue haemorrhagic fever. In *WHO Report on Global Surveillance of Epidemic-prone Infectious Diseases*. Geneva: World Health Organization. Available at: http://www.who.int/emc-documents/surveillance/docs/whocdscsisr2001.html/dengue/dengue.htm.

Winter, P.E., Yuill, T.M., Udomsakdi, S., Gould, D., Nantapanich, S. and Russell, P.K. (1968) An insular outbreak of dengue hemorrhagic fever. I. Epidemiologic observations. *Am J Trop Med Hyg* 17: 590–599.

Yauch, L.E. and Shresta, S. (2008) Mouse models of dengue virus infection and disease. *Antiviral Res* 80: 87–93.

Young, N.S. (1990) Flaviviruses and bone marrow failure. *JAMA* 263: 3065–3068.