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REVIEW ARTICLE

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Dengue illness: clinical features and pathogenesis

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Abstract The incidence and geographical distribution of dengue has gradually increased during the past decade. This review is an update on dengue virus infections, based on our clinical and laboratory experiences in the Philippines and on other relevant literature. The differential diagnosis of this disease is discussed, especially for use by clinicians where dengue is not endemic. The complex pathogenesis of thrombocytopenia and increased vascular permeability in dengue illness is also discussed. Our recent data suggest that platelet-associated immunoglobulins involving anti-dengue virus activity play a pivotal role in the development of dengue hemorrhagic fever (DHF), as well as thrombocytopenia in secondary dengue virus infections. Further elucidation is needed on the involvement of platelet-associated immunoglobulins on the molecular mechanisms of thrombocytopenia and the increased vascular permeability.

Key words Dengue fever · Dengue hemorrhagic fever · Thrombocytopenia · Increased vascular permeability

Introduction

Dengue virus, a mosquito-borne human viral pathogen, belongs to the genus *Flavivirus* of the family *Flaviviridae* (single-strand, nonsegmented RNA viruses), and has four serotypes (DEN-1, DEN-2, DEN-3, and DEN-4).[1] Infection with one serotype confers immunity to the infected serotype for a long period, but not to other serotypes. Humans may therefore be infected with the dengue virus up to four times. The transmission cycle of dengue viruses involves the *Aedes* mosquito and lower primates in the rain forests of Asia and Africa.[1] These viruses do not move out of the forest into urban areas, but an epidemic transmission cycle in rural villages and islands may be possible. A number of *Aedes* mosquitoes, such as *Aedes aegypti* and *A. albopictus*, may act as a vector in these situations. The most important transmission cycle is the urban epidemic cycle in tropical and subtropical countries. The dengue virus is now the most frequent cause of arboviral diseases world-wide, and it has become a major public health concern, particularly in these areas.

The global epidemiology and the dynamics of transmission of dengue viruses have changed dramatically in Southeast Asia since World War II.[2] The change in the ecology caused by the war expanded the geographical distribution of *Aedes* mosquitoes, and urbanization and modern transportation after World War II contributed greatly to the increased incidence of dengue activity. The disease is now highly endemic in more than 100 tropical countries (Fig. 1), and the number of cases has increased dramatically during the past three decades.[2–4] Figure 2 shows the annual number of dengue fever (DF)/dengue hemorrhagic fever (DHF) cases and deaths reported to the World Health Organisation (WHO) during the period 1969–2003.[5] Although the number of deaths has decreased recently, the case numbers of DF/DHF have gradually increased during the past decade. Major epidemics occurred in Asian and American countries, with more than 1.2 million cases of DF/DHF in 1998. Two and a half billion people are now at risk of infection, with an estimated 50–100 million cases of DF and several hundred cases of DHF per year.

The purpose of this review article is to update our current knowledge of dengue virus infections, and focus on the differential diagnosis of this disease, and the complex pathogenesis of thrombocytopenia and increased vascular permeability associated with it.

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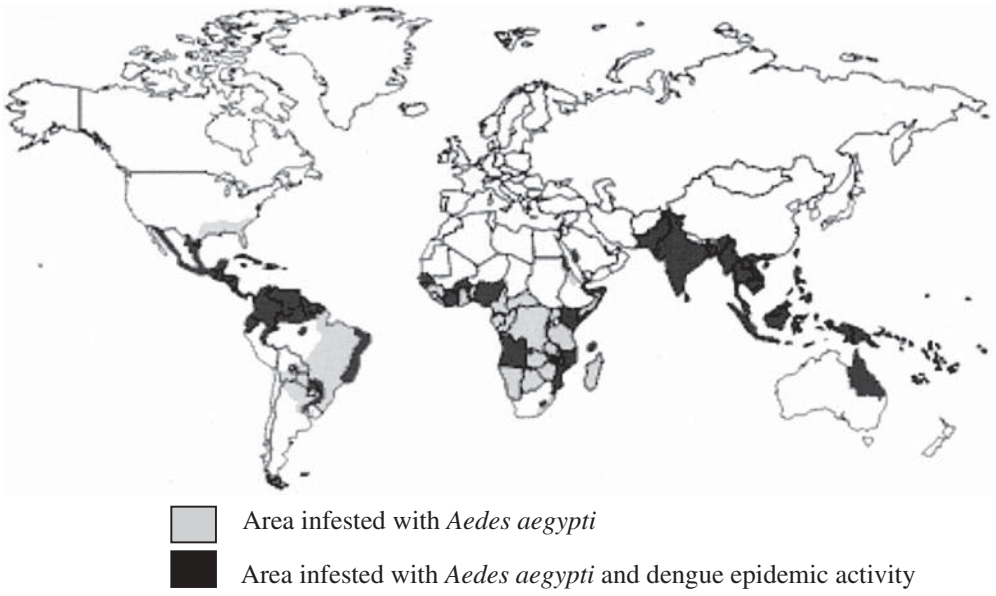

Fig. 1. World-wide distribution of dengue viruses and their mosquito vector *Aedes aegypti* in 2003

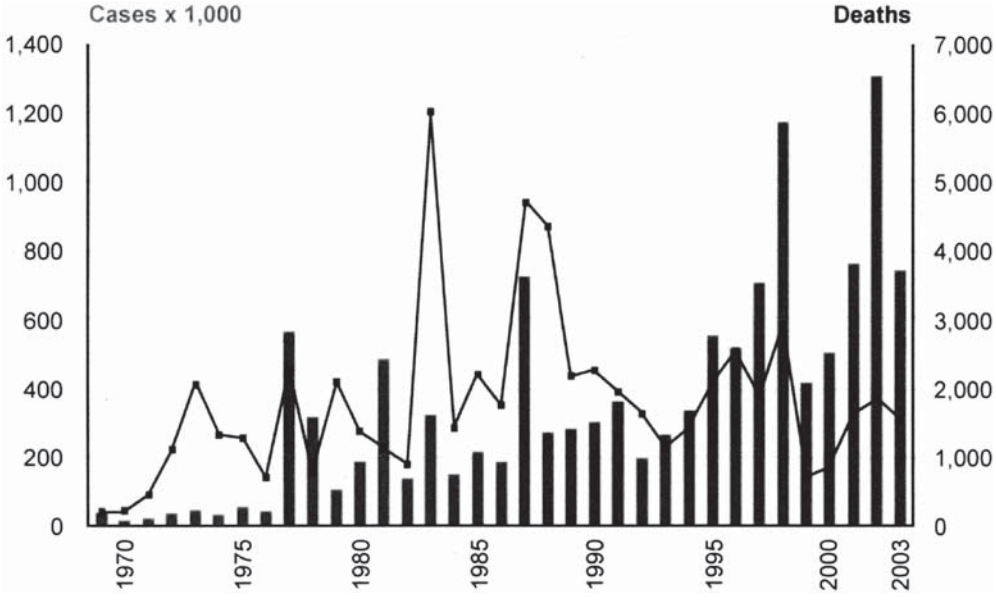

Fig. 2. Annual number of dengue fever/dengue hemorrhagic fever (DF/DHF) cases and deaths reported to the World Health Organisation (WHO), 1969–2003. *Lines* indicate the number of deaths, and *bars* indicate the number of cases per year

Clinical manifestations and management

Dengue virus infections cause a spectrum of illnesses ranging from asymptomatic to DF and DHF. While DF is a self-limited febrile illness, DHF is often characterized by prominent hemorrhagic manifestations associated with an increased vascular permeability. After an infected mosquito has bitten a human, the virus replicates in the regional lymph nodes and is then disseminated into the blood circulation and other organs.[1,4] The incubation period is typically 4–7 days.

DF is characterized by the sudden onset of fever and other nonspecific symptoms, such as headaches. The fever may continue for 5–7 days. Approximately 50% of patients have skin problems.[6] While flushed faces are usually seen during the first 24–48 h, a petechial rash or maculopapular rash can frequently be seen during the period of defervescence (Fig. 3). Laboratory abnormalities involve thrombocytopenia, leukocytopenia, and increased levels of hepatic aminotransferase.[7] The clinical course of an imported case of DF is shown in Fig. 4.[8] A 21-year-old student visited the Solomon Islands between August 19 and September 2, 2001. When the student returned to Japan, he was immediately admitted to hospital, on September 5, with a high fever that persisted for 2 days. A physical examination on September 5 showed clear consciousness, a high fever (body temperature 38.4°C), normal blood pressure, and hyperemic conjunctiva. No hemorrhage in the nose and gingiva, and no skin rash were observed. The result of IgM-capture ELISA was negative on the day of admission, but reverse transcriptase–polymerase chain reaction (RT-PCR) for

DEN-2 was positive on September 6. The patient became afebrile, and the IgM-capture ELISA became positive, and he developed minimal nasal bleeding and petechial rash on September 10. Thrombocytopenia was found around the day of defervescence. Leukocytopenia and elevated aspartate aminotransferase (AST) levels were also found during the acute phase of the illness (Fig. 4).

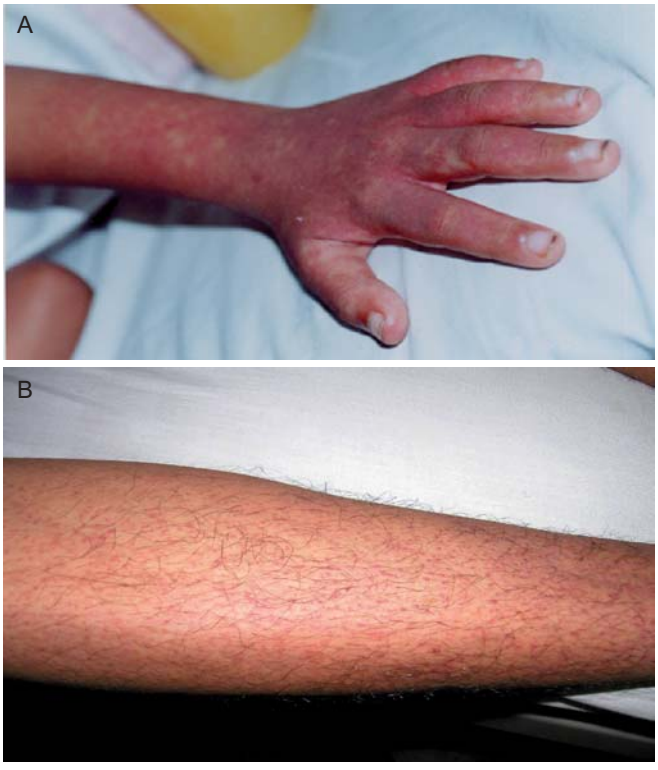

Fig. 3. Morbilliform rash with sparing of islands of the skin (**A**) and a petechial rash (**B**) found in a patients with dengue fever

DHF is associated with severe thrombocytopenia and capillary plasma leakage, especially during the period of defervescence. The diagnostic criteria of DHF include a platelet count nadir of less than 100000/μl, hemorrhagic manifestations and an increase in hematocrit greater than 20% above the average, or the presence of pleural effusion or ascites.[9] Cases of DHF are further graded as I–IV. DHF grades III and IV are dengue shock syndrome, which is caused by increased plasma leakage, and is clinically characterized by a rapid, weak pulse with a narrowing pulse pressure of less than 20 mmHg or profound hypotension. No specific treatments are available for dengue. The prompt and correct institution of fluid replacement is thought to reduce mortality rates due to DHF/DSS. The case fatality rate of DF/DHF is typically less than 0.5% under appropriate management in hospital, but it can range as high as 10%–20%.[1,2]

Laboratory diagnosis

A laboratory diagnosis of dengue virus infection is established by the detection of the dengue virus-specific antibody, or detection of the genomic sequence by RT–PCR or viral isolation.[10] Serological detection based on IgM-capture ELISA and IgG ELISA or a hemagglutination inhibition test have become the new standards for the detection and differentiation of primary and secondary dengue virus infections. IgM-capture ELISA may be negative in the early phase of acute illness (6 days after onset). RT–PCR, which has the advantage of detecting dengue virus in acute-phase

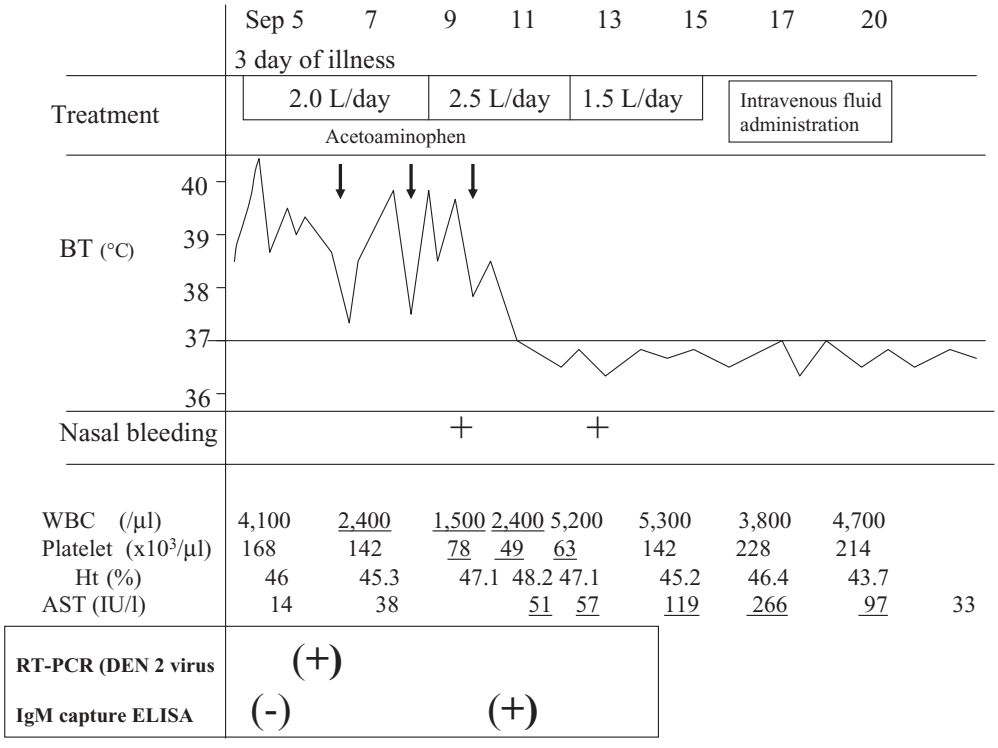

Fig. 4. Clinical course of a Japanese traveler, returning home with dengue fever. *BT*, body temperature; *WBC*, white blood cells; *AST*, aspartate aminotransferase; *RT–PCR*, reverse transcriptase-polymerase chain reaction; Ht, hematocrit

serum, represents a method for the diagnosis and differentiation of the four dengue virus serotypes. Because the diagnostic sensitivity range is 90%–93% in IgM-capture ELISA and 80%–100% in RT–PCR, the diagnostic sensitivity of RT–PCR in combination with IgM-capture ELISA will be higher than 90%.[11–13] More recently, a fully automated real-time PCR assay has become available for the detection or quantification of the dengue virus using acute-phase serum. The real-time PCR assay has a variety of advantages over conventional RT–PCR, and provides a quantitative measurement of viral titers with a lower contamination and a high sensitivity. This method also appears to be useful for investigating the pathogenesis of dengue illness,[14] and may gradually replace conventional PCR as the gold standard for a rapid laboratory test for dengue virus infections.

Differential diagnosis

A flowchart of the management of a febrile returning traveler is shown in Fig. 5. When a febrile patient shows some hemorrhagic manifestations, it may not be difficult to diagnose dengue illness. The combination of these clinical signs and laboratory data is highly predictive of a diagnosis of this disease. If no hemorrhagic manifestations are present, it is important to rule out malaria until another diagnosis is confirmed or the patient has improved. When malaria is ruled out and no localized findings are present, the differential diagnosis for a febrile patient includes typhoid fever, brucellosis, leptospirosis, rickettsial diseases, viral diseases such as measles, mumps, chikungunya, and other viral hemorrhagic fevers, and other parasitic infections.[4,5,15]

In order to assist an understanding of the laboratory data that are characteristic for dengue illness, comparative laboratory data for white blood cell counts (WBC), platelet counts, and the hematocrit (Ht) among pediatric patients with DF, DHF, and other febrile illnesses (OFI) are shown in Fig. 6A–C, respectively. A total of 503 subjects, who were screened for acute febrile illness without an apparent focus of infection, were admitted to St. Luke's Medical Center (SLMC), Quezon City, Philippines, between January 1999 and December 2001, and the clinical records of those who satisfied the following criteria were studied: (1) age between 2 and 17 years; (2) fever for ≤5 days; (3) body temperature of at least 37.8°C; (4) no apparent focus of infection.[16] Of the 503 patients who were screened, 359 (71.4%) were confirmed as having a dengue virus infection by IgM-capture ELISA or RT–PCR. Of the 359 laboratory-confirmed cases, 239 (66.6%) and 120 (33.4%) were diagnosed as DF and DHF, respectively, based on WHO criteria.[8] The other 144 screened cases (28.6%) were diagnosed as OFI. Although most of the cases with OFI (79.2%) were undiagnosed, 20 (13.9%) were diagnosed with acute lower respiratory infections. Of these, 7 were diagnosed with radiological pneumonia. Other illnesses, such as acute gastroenteritis, meningitis, measles, typhoid fever, and urinary tract infections were also involved. Patients with OFI (mean age 8.6 years) were significantly younger than those with DF (mean age 9.9

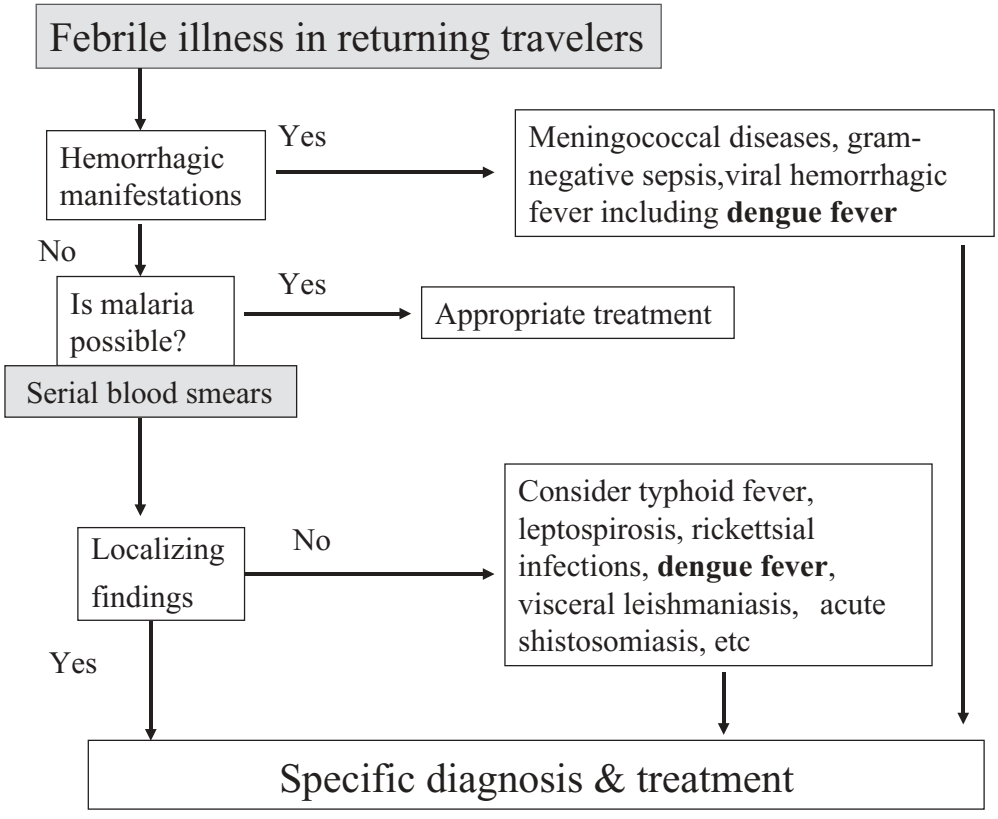

Fig. 5. Flowchart for the management of a returning traveler

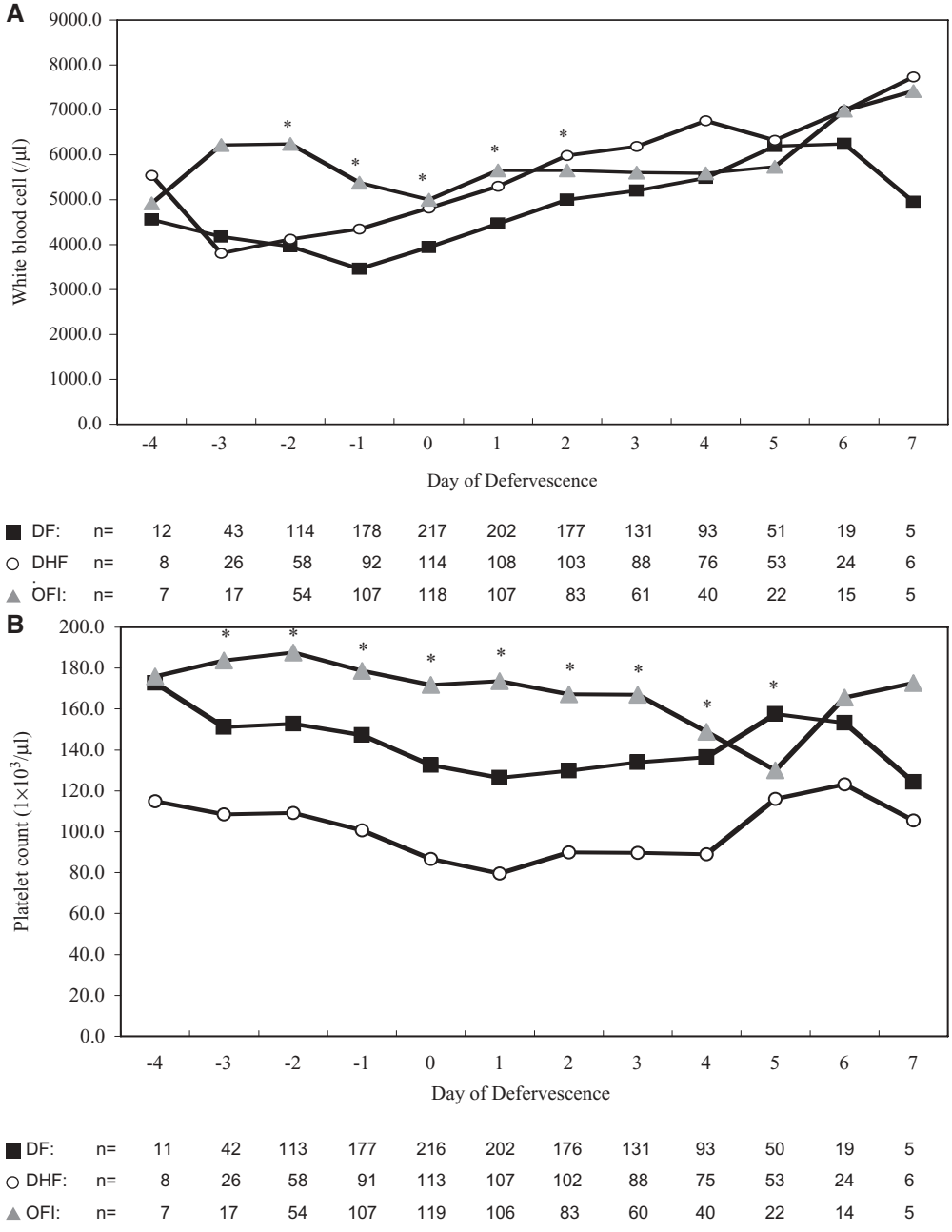

Fig. 6. Comparison of total white blood cells (**A**), platelet count (**B**), and the hematocrit (**C**) in peripheral blood among pediatric patients with dengue fever (*DF*), dengue hemorrhagic fever (*DHF*) and other febrile illness (*OFI*). The number of cases with *DF*, *DHF*, and *OFI* are shown below each figure. *Open circles* show cases of *DF*, *filled squares* show cases of *DHF*, and *gray triangles* show cases of *OFI*. The number of days before and after defervescence are shown consecutively as follows: –2, –1, 0, +1, +2, etc. Data represent the means. The numbers were analyzed by the post hoc multiple comparison test using the Bonferroni method. **A** Day –2: $P < 0.001$ (*DF* vs *OFI*), $P = 0.005$ (*DHF* vs *OFI*); Day –1: $P = 0.010$ (*DF* vs *DHF*), $P < 0.001$ (*DF* vs *OFI*), $P = 0.006$ (*DHF* vs *OFI*); Day 0: $P = 0.018$ (*DF* vs *DHF*), $P = 0.002$ (*DF* vs *OFI*); Day 1: $P = 0.006$ (*DF* vs *OFI*); Day 2: $P = 0.029$ (*DF* vs *DHF*). **B** Day –3: $P = 0.001$ (*DHF* vs *OFI*), Day –2: $P < 0.001$ (*DF* vs *DHF*), $P = 0.005$ (*DF* vs *OFI*), $P < 0.001$ (*DHF* vs *OFI*); Day –1: $P < 0.001$ (*DF* vs *DHF*), $P < 0.001$ (*DF* vs *OFI*), $P < 0.001$ (*DHF* vs *OFI*); Day 0: $P < 0.001$ (*DF* vs *DHF*), $P < 0.001$ (*DF* vs *OFI*), $P < 0.001$ (*DHF* vs *OFI*); Day 1: $P < 0.001$ (*DF* vs *DHF*), $P < 0.001$ (*DF* vs *OFI*), $P < 0.001$ (*DHF* vs *OFI*); Day 2: $P < 0.001$ (*DF* vs *DHF*), $P < 0.001$ (*DF* vs *OFI*), $P < 0.001$ (*DHF* vs *OFI*); Day 3: $P < 0.001$ (*DF* vs *DHF*), $P = 0.014$ (*DF* vs *OFI*), $P < 0.001$ (*DHF* vs *OFI*); Day 4: $P < 0.001$ (*DF* vs *DHF*), $P < 0.001$ (*DHF* vs *OFI*); Day 5: $P = 0.015$ (*DF* vs *DHF*). **C** Day –3: $P = 0.002$ (*DHF* vs *OFI*); Day –2: $P = 0.043$ (*DF* vs *DHF*), $P < 0.001$ (*DHF* vs *OFI*); Day –1: $P = 0.002$ (*DF* vs *OFI*), $P < 0.001$ (*DHF* vs *OFI*); Day 0: $P = 0.005$ (*DF* vs *OFI*), $P < 0.001$ (*DHF* vs *OFI*); Day 4: $P = 0.034$ (*DF* vs *DHF*)

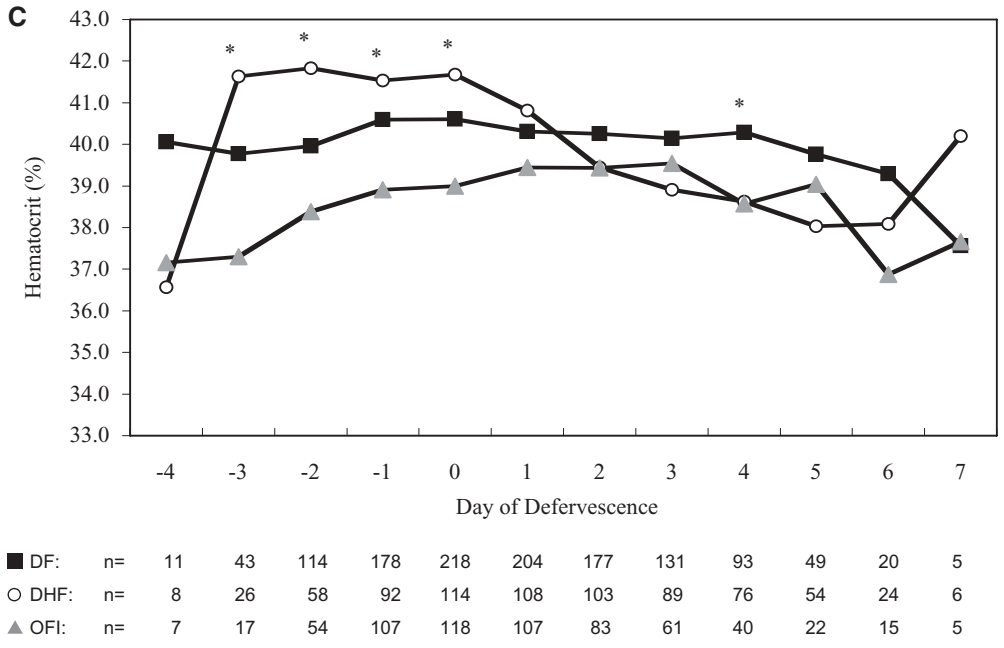

Fig. 6. *Continued*

years) or DHF (mean age 9.8 years). Peripheral white blood cell counts (WBC) were significantly lower in patients with DF or DHF than in patients with OFI around the day of defervescence (Fig. 6A). The peripheral platelet counts were also significantly lower in patients with DF or DHF than in patients with OFI from 3 days before to 4 days after the day of defervescence (Fig. 6B). Ht values were significantly higher in patients with DHF than in those with DF from day –3 to day +0 (Fig. 6C). The Ht was also significantly higher in patients with DF than in patients with OFI at days –1 and +0. A transient increase in vascular permeability shown by an increased Ht is characteristic of DHF. These laboratory data, which are characteristic of patients with DF or DHF, can be helpful for the differential diagnosis of dengue illness.

Pathogenesis

The hallmarks of dengue virus infections are hemorrhagic manifestations associated with thrombocytopenia and an increased vascular permeability, which is the main feature of DHF. Plasma leakage into the serous space is obvious in most cases of DHF.

Thrombocytopenia

A previous report suggested that dengue virus-induced bone marrow suppression decreases platelet synthesis, resulting in thrombocytopenia.[17] In addition, we previously reported that coagulation abnormalities involve a combination of thrombocytopenia and increased fibrinolysis, but not classic disseminated intravascular coagulation (DIC), in the majority of patients with dengue virus infections.[16] On the other hand, a study of platelet kinetics in patients with dengue hemorrhagic fever indicated an increase in platelet destruction as the major cause of thrombocytopenia.[18] A recent investigation reported the presence of an anti-platelet autoantibody in sera from patients infected with dengue virus and thrombocytopenia.[19] Another study demonstrated that both the dengue virus antigen and human immunoglobulins are present on the platelet surface in patients with a dengue hemorrhagic fever.[20] Thrombocytopenia due to increased platelet destruction by an immune mechanism may therefore be operative among dengue patients, although the precise mechanism(s) for the development of thrombocytopenia remain unclear. An increased level of PAIgG is frequently observed in patients with chronic idiopathic thrombocytopenic purpura (ITP), but is also found in a variety of other diseases.[21–24] Although virus-associated ITP, such as a human herpes virus 6 infection, has also been recognized,[25,26] the association between increased levels of PAIgG and the mechanisms of PAIgG-mediated thrombocytopenia has not been examined extensively in these viral infections. We recently demonstrated an inverse correlation between the levels of platelet-associated IgG (PAIgG) and the platelet count during the acute phase of secondary dengue infections.[27] Circulating anti-platelet autoantibody was rarely detected in this study. We speculated that immune complexes of the dengue virus with anti-dengue virus IgG antibodies are located on the platelet, as a result of the direct binding of the dengue virus to platelets.[28] The findings shown in this study suggest that PAIgG formation, involving anti-dengue virus IgG, plays an important role in inducing thrombocytopenia in secondary infections (Fig. 7). Because thrombocytopenia is more prominent in DHF than in DF,[7,16] we hypothesized that the increased level of PAIgG as well as PAIgM might be associated with the severity of the disease as well as the accompanying thrombocytopenia.

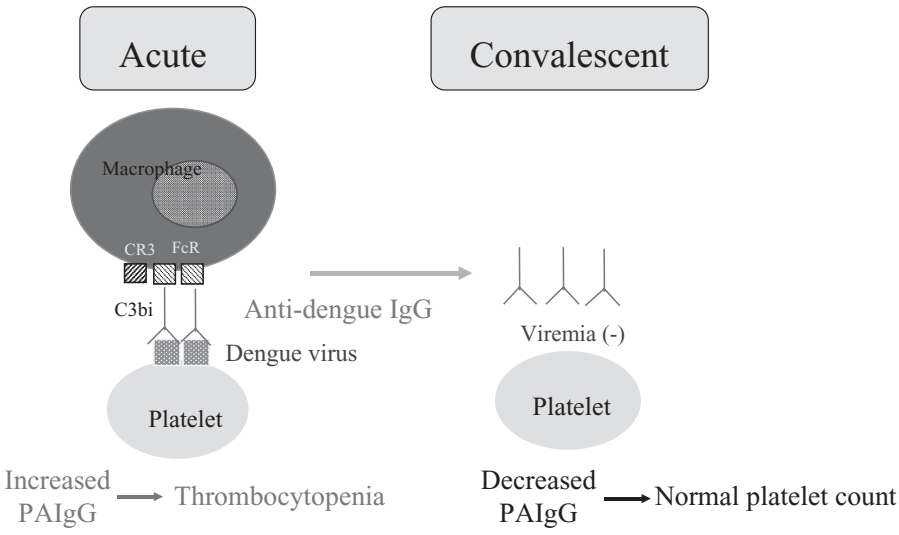

Fig. 7. A possible mechanism for thrombocytopenia through the formation of platelet-associated IgG (PAIgG) in secondary dengue virus infections. During the acute phase of viremia, immune complexes of dengue virus with anti-dengue virus IgG antibodies, which are located on platelets via the direct binding of dengue virus to platelets, and PAIgG formation may result in thrombocytopenia due to platelet clearance by macrophages and/or complement-mediated platelet lysis

We therefore conducted a prospective hospital-based study among patients with secondary dengue virus infections.[29] In this study, an inverse correlation between platelet count and PAIgG or PAIgM levels was found in these patients. Anti-dengue virus IgG and IgM activity was found in platelet eluates from DF or DHF patients in the acute phase of a secondary infection. While PAIgG formation may induce thrombocytopenia through both Fc receptors and complement receptor-mediated platelet clearance by macrophages and/or complement-mediated platelet lysis,[21,30,31] PAIgM formation may also result in thrombocytopenia via the same mechanisms except for Fc receptors, based on the function of the IgM pentamer. We also demonstrated that the levels of PAIgG or PAIgM were significantly higher in DHF than in DF.[29] Collectively, our data suggest that platelet-associated immunoglobulins involving anti-dengue virus activity play a pivotal role in the development of DHF, as well as thrombocytopenia in secondary dengue virus infections. Further studies of the involvement of platelet-associated immunoglobulins on the molecular mechanisms of thrombocytopenia are now being developed in the Philippines.

Increased vascular permeability

Because tissue damage is not prominent compared with the severity of the diseases, altered vascular permeability, but not the structural destruction of endothelial cells, contributes to the development of DHF. Although the mechanisms of DHF development are not completely understood, two of the hypotheses for explaining the pathogenic changes of DHF on the basis of epidemiological data are (1) the secondary heterotypic antibody-dependent enhancement of a dengue virus infection, which is widely accepted,[32–34] and (2) a combination of viral load, strain virulence, and host immune response.[14,35] Although the dengue virus infects different cells, such as Kupffer cells, alveolar macrophages, vascular endothelial cells, monocytes, and lymphocytes,[36] cells from the monocyte lineage, such as Langerhans cells in the skin and interstitial dendritic cells (DCs), are the primary viral targets.[37] Certain subsets of DC which are susceptible to the dengue virus express DC-specific intracellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), and this molecule has been reported to be essential for dengue virus infections.[38]

Secondary infections, which are commonly observed in dengue endemic areas, are more likely to constitute a risk factor for DHF.[33,34] More than 90% of DHF cases occur during secondary infections. Cross-reactive nonneutralizing anti-dengue virus antibodies form complexes with heterologous dengue viruses, and these virus–antibody complexes facilitate viral uptake into monocytes via the Fc-receptor.[39–41] This process is known as antibody-dependent enhancement (ADE). The Fc receptor of anti-dengue virus IgG and FcγR on cells are necessary for this to occur.[42] Dengue virus-specific memory CD4+CD8– and CD4-CD8+ T cells are induced after an infection with the dengue virus.[43] It is hypothesized that a positive feed-back loop exists in which ADE increases the number of antigen-presenting cells that stimulate dengue cross-reactive memory CD4+ and CD8+ T cells. The activation of monocytes, T cells, and DC induce the production of cytokines and chemical mediators, which may cause capillary leakage that leads to shock in DHF cases.[44,45] These cytokines include IL-2, IFN-γ, and TNF α, and chemokines such as IL-8 and MCP-1.[44–50] The dengue virus, therefore, through an indirect more than a direct mechanism, could mediate endothelial cell activation. The role of TNF α in the pathogenesis of the disease is critical, and probably initiates several processes that are related to plasma leakage.[51,52] In addition, a recent study reported that inflammatory endothelial activation is induced by the anti-dengue virus nonstructural protein (NS)-1 via the NF-κB pathway.[49] Cytokine production was enhanced in the endothelial cells in the presence of the anti-NS-1 antibody. Furthermore, complement activation as a result of immune complexes or immune activation could also be involved in the mechanism of increased vascular permeability.[30,31] NS-1 induces complement activation, which is enhanced by an anti-NS-1 antibody. On the other hand, the infection of endothelial cells with the dengue

virus induces apoptosis.[47] Complement activation and the apoptosis of endothelial cells by dengue virus infections may lead to increased vascular permeability. The clearance of dengue virus-infected apoptotic endothelial cells may also explain the abrupt termination of a dengue virus infection and the increased vascular permeability. A recent report has also suggested that the overproduction of matrix metalloproteinase (MMP) 9 by immature DC infected by the dengue virus is involved in the enhanced endothelial permeability.[53]

Dengue in international travelers

During the past few decades, increasing numbers of international travelers with dengue have been reported from countries where dengue is now endemic. DF has been diagnosed in an increasing proportion (ranging from 2% to 16% in recent years) of febrile travelers returning from the tropics.[54,55] Most dengue virus infections in travelers are acquired in Asia, followed by Central and South America, with only a small proportion in Africa. A recent study reported that the proportionate morbidity is approximately 80 per 1000 ill travelers returning from Southeast Asia, followed by 40 from the Caribbean, and 20 from Central and South America.[56] Furthermore, the frequency of dengue infection in travelers is higher than malaria for every region except sub-Saharan Africa. In Japan, approximately 50 imported cases of dengue fever per year have been reported between 2000 and 2005 by the National Institute of Infectious Disease, Japan. Most of the cases were from Southeast and South Asian countries and the Pacific Ocean. A recent study by Japanese investigators reported 62 imported cases of DF.[57] Most of these patients were also returning from Asian countries.

Effective control of dengue is difficult to achieve. Public health efforts should be focused on vector control, but this is difficult because *Aedes* mosquitoes are frequently in close contact with humans. The single most effective preventive measure for travelers is the use of insect repellents containing DEET (N, N, diethyl-meta-toluamide) and a powerful, rapid-acting insecticide, permethrin.[14] A combination of permethrin-treated clothing and the application of a DEET-based repellent to the skin creates a formidable barrier against mosquitoes. Because *Aedes* mosquitoes are active and bite during the day, these measures should be taken during the day. The production of live, attenuated, tetravalent dengue vaccines is now under development, and these vaccines are expected to confer protective immunity against the four serotypes of the dengue virus without antibody-dependent enhancement[58] Pretravel advice is also important for travelers. This information should include the risk of dengue, destinations associated with high risk, and signs and symptoms of this disease. Although the incidence of dengue is highest during the rainy season, cases have also been found during the nonrainy season. More importantly, the risk of exposure also exists in urban and residential areas.

Dengue virus infection will continue to spread throughout the world until effective vector control is achieved and cost-effective vaccines become available. In areas where dengue is not endemic, clinicians should be aware of the clinical manifestations and management of this disease, and travelers should be well aware of the risks of dengue and preventive measures in dengue-endemic countries.

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