MINIREVIEW

Alternate Hypothesis on the Pathogenesis of Dengue Hemorrhagic Fever (DHF)/ Dengue Shock Syndrome (DSS) in Dengue Virus Infection

SANSANEE NOISAKRAN,*,† AND GUEY CHUEN PERNG‡,[1]

*Medical Biotechnology Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani, 12120, Thailand; †Medical Molecular Biology Unit, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand; and ‡Department of Pathology and Laboratory Medicine, Emory Vaccine Center, School of Medicine, Emory University, Atlanta, Georgia 30322

Dengue fever, caused by infection with dengue virus, is not a new disease, but recently because of its serious emerging health threats, coupled with possible dire consequences including death, it has aroused considerable medical and public health concerns worldwide. Today, dengue is considered one of the most important arthropod-borne viral diseases in humans in terms of morbidity and mortality. Globally, it is estimated that approximate 50 to 100 million new dengue virus infections occur annually. Among these, there are 200,000 to 500,000 cases of potential life-threatening dengue hemorrhagic fever (DHF)/ dengue shock syndrome (DSS), characterized by thrombocytopenia and increased vascular permeability. The death rate associated with the more severe form DHF/DSS is approximately 5%, predominantly in children under the age of 15. Although intensive efforts have been made to study the early clinical pathophysiology of dengue infection with the objective to identify the potential cause of DHF, results or data that have accumulated from different regions of the world involving studies of different ethnicity groups are inconsistent at present in terms of identifying a unified hypothesis for the pathogenesis of DHF/DSS. Thus, the potential mechanisms involved in the pathogenesis of DHF and DSS remain elusive. The purpose of this review is to identify alternate factors, such as innate immune parameters, hyper-thermal factors, conditioning of neutralizing antibody, concept of vector transmission, and physical status of virus in viremic patients that may play a role in the induction of DHF and DSS, which might have directly or indirectly contributed to the discrepancies that are noted in the literature reported to date. It is the hope that identification of an alternative explanation for the pathogenesis of DHF/DSS will pave the way for the institution of new strategies for the prevention of this complicated disease. Exp Biol Med 233:401–408, 2008

Key words: Flavivirus; DHF; DSS; emerging infectious disease

Introduction

Dengue Virus. The dengue viruses, the cause of dengue illness, are members of the Flaviviridae family. Four genetically related but distinct serotypes, designated DENV-1, DENV-2, DENV-3, and DENV-4, are circulating worldwide (1). The main vector for dengue virus transmission is the *Aedes aegypti* species of mosquitoes. Dengue viruses, similar to other flaviviruses, have a positive single-stranded RNA genome packaged inside a core protein, which is surrounded by an icosahedral scaffold and covered by a lipid envelope (2). The genome of the dengue virus contains an 11-kb plus-sensed RNA encoding 3 structural proteins and 7 nonstructural proteins (Fig. 1). Viral protein and RNA synthesis occur predominantly in the cytoplasm of host cells (3). Replication is slow and begins within 15 hrs after infection. Low amounts of dengue virus are released into the supernatant fluid. Dengue virus replication does not

[1] To whom correspondence should be addressed at Department of Pathology and Laboratory Medicine, Emory Vaccine Center, School of Medicine, Emory University, Dental Building Room 429, 1462 Clifton Road, Atlanta, GA 30322. E-mail: gperng@emory. edu

Received July 24, 2007.
Accepted October 14, 2007.

DOI: 10.3181/0707-MR-198
1535-3702/08/2334-0401$15.00

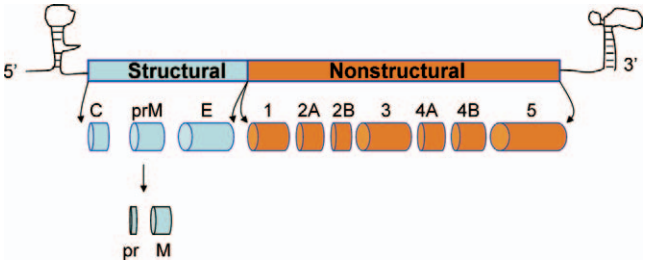

Figure 1. The dengue viral genome. Noncoding regions with their terminal structures are indicated by black lines. The single open reading frame encodes a poly-protein that is processed by the viral NS2B-NS3 protease and cell proteases to the mature viral proteins. Structural proteins are C, prM, and E. Nonstructural proteins are 1, 2A, 2B, 3, 4A, 4B, and 5. The genome is not drawn to scale. A color figure is available in the online version.

significantly affect the metabolic function of the host cell as exemplified by normal levels of protein synthesis by the infected host cells (3).

Epidemiology of Dengue Infection. Dengue infections are at present one of the most common mosquito-borne viral diseases of humans worldwide. Initially, dengue infections were primarily recorded when they occurred as epidemics in tropical and subtropical countries. But over time, increasing globalization and human movement coupled by the increase in the geographic area where the *Aedes aegypti* mosquito vector inhabit, has promoted dengue virus infection to nearly every corner of the world (4, 5). The National Institute of Allergy and Infectious Diseases (NIAID) has listed dengue virus as a category A priority biothreat pathogen (6). Approximately 50 to 100 million people contract dengue fever annually, and about 200,000 to 500,000 contract dengue hemorrhagic fever (DHF), and the mortality rate is about 5%, predominantly in children under 15 years of age (7). The pathogenesis of DHF and dengue shock syndrome (DSS) is poorly understood. Epidemiologically, secondary infections, which occur commonly in dengue-endemic areas, have correlated well with the occurrence of severe dengue viral illness and therefore are considered as one of the major risk factor for severe dengue disease (8–10). This view led to the advancement of the antibody-dependent enhancement theory three decades ago (11). Other notable risk factors for DHF include the strain/serotype of the infecting virus, age of the patient, and the genetic background of the patient (2, 12). Recently, evidence has been presented that suggests that not only dengue virus serotypes, but flaviviruses in general may share peptide sequences, which serve as immunodominant determinants. T cells that recognize such determinants once primed during primary infection with one of the dengue virus serotype or a member of the Flavivirus family respond vigorously (memory T-cell responses) upon exposure to a second infection releasing massive amounts of proinflammatory cytokines, which induce vascular endothelial cell activation and are the likely cause of the capillary leak syndrome and the basis of DHF pathogenesis (13–15). While this concept emerged specially from studies of mice immunized with recombinant proteins of the dengue or Flavivirus, evidence for its occurrence in the clinical setting continues to be a subject of intense debate.

Clinical Manifestations of Dengue Disease. Each of the four dengue virus serotypes is capable of causing a spectrum of diseases ranging from mild infection to a potentially deadly disease. Infection with one serotype leads to life-long immunity to that serotype but only partial and temporary immunity to the others (16). As outlined above, circulation of more than one serotype upon infection can increase the risk of serious and complicated infections, i.e., DHF and DSS (10). Infection with any serotype can be asymptomatic or lead to one of the four clinical scenarios of increasing severity: undifferentiated fever, dengue fever, DHF, and DSS (1, 17).

Dengue Fever. Dengue fever (DF) follows the bite of a mosquito carrying infectious dengue virus. DF is an acute and a self-limited dengue disease, and is a syndrome that is associated with the occurrence of fever that lasts from 2 to 7 days, headache, myalgia, bone/joint pain and rash, often accompanied by leucopenia. Occasionally variable degrees of thrombocytopenia and cutaneous hemorrhage are observed. More severe cases with incapacitating bone/joint pain ("break-bone-fever") are common among adults. Infrequently, DF may be accompanied by unusual bleeding complications that may cause death (18).

Dengue Hemorrhagic Fever. The clinical features of DHF in many aspects are very similar to that of DF during the early febrile phase. The prominent feature of DHF is its potential to develop into fatal DSS. The major pathophysiologic hallmarks that determine disease severity and distinguish DHF from DF are plasma leakage as a result of increased vascular permeability and abnormal hemostasis, occurring in a select group of patients during the course of dengue infection (18). The underlying mechanisms causing DHF/DSS are a subject of intense debate. Current evidence strongly suggests that the intensity of the immune response to dengue virus plays a key role in the pathophysiologic cascade leading to plasma leakage (19–21). In addition, DHF and DSS have been associated with high levels of proinflammatory cytokines in the serum of patients (22–26). In addition, other mediators produced by phagocytic cells and a role for antibody mimicry have been suggested (27–29).

Hypotheses on the Pathogenesis of DHF/DSS. There are numerous theories on how DHF/DSS develops in infected dengue individuals. These hypotheses are predominantly derived from data obtained on studies conducted within the regions of countries where the disease occurs in an epidemic form, and/or to some extent, from *in vitro* experiments. These include antibody-mediated pathogenesis or so-called antibody-dependent enhancement, cell-mediated pathogenesis (10), cytokine storm phenomenon (30), individual's genetic background (31), virus strain differences (32, 33), levels of virus circulating in individuals during the acute phase (34, 35), and the nutritional status of the infected individual (36). The major limitations in our

ability to decipher or dissect the mechanisms of pathogenesis of dengue virus infection are a lack of suitable small animal models. Consequently, the precise mechanisms that lead to the development of DHF/DSS remain an enigma.

In addition to the aforementioned hypotheses, the authors reason that other factors, which are closely associated with dengue virus infection, have not been studied in sufficient detail. These include (i) hyper-thermal factors, (ii) physical status of virus in viremic individuals, (iii) conditioning of neutralizing antibody assay in dengue virus infection, (iv) concept of vector transmission, and (v) innate immune system. We, therefore, offer that detailed studies of these events that occur during the acute disease stage are critical in shedding light on the pathogenesis of DHF/DSS. A detailed understanding of these events may provide an alternate scenario in which these factors may contribute directly or indirectly to DHF/DSS and are the subject of this review.

(i) Hyper-thermal Factors. As the name of the disease implies, dengue fever is one of the hallmarks of dengue virus infection. However, there has been limited attention given to the distinguishing characteristics of this fever that occur in patients who simply develop DF as compared with those that develop DHF or DSS.

The period of fever following dengue virus infection lasts from 2 to 7 days. Fever is the normal physiologic response of the body to mediators that are generated during the acute phase of dengue virus infection. In fact, fever is a condition induced by the host to subdue and contain the adverse effects of the infection, making it unfavorable for the infecting agent, and is designed to promote body health. Heat-inducible factors from host cells are expressed and released into the circulation. Most prominent amongst these heat inducible factors are cytokines that trigger fever (fever inducers), such as TNF-alpha, IL-1 and IL-6, and cytokines to calm down fever (fever inhibitors), such as TGF-beta and IL-10 (37). These cytokines are promiscuous effectors and can be found in febrile patients as a response to a variety of infectious agents, including dengue virus infections. Differences in the quality and/or quantity of these array of fever-related factors circulating in the blood may alter or enhance parameters observed in dengue virus–infected patients and may be critical in defining the outcome of such an infection. In this regard it is of interest to note that hyper-thermal conditions have in fact been reported to increase cell susceptibility to virus infection (38–40). Thus, a more careful analysis of the spectrum and quantitation of cytokines that either induce or are secondary to the thermal response following dengue virus infection may be critical in identifying the possible factors or mechanisms that may distinguish DF from DHF or DSS and shed light on the pathogenesis of DHF/DSS.

(ii) Physical Status of Virus in Viremic Individuals. Viremia is the major unique feature in dengue virus infection among the Flavivirus family. Although this feature is accepted and well-known, the precise nature of this viremia remains to be established. Thus, physical evidence on whether the virus exists as a free viral particle, or is primarily cell associated and/or is encapsulated by host cell membrane, remains unknown.

Viremia can be caused by the presence of the virus in plasma (free viral particle) or be cell associated, such as within platelets, lymphocytes, monocytes, but not likely within red blood cells. In the case of plasma viremia, the amount of circulating virus is a critical factor for its clearance. Results of one study suggest that most of the virus circulating in the blood is cell-associated (41). These cell-associated viruses are poorly cleared when injected into a normal host, as compared with the clearance of plasma associated virus that is 95% cleared in less than 2 mins (41). Of importance is the finding that the cell-associated virus by its nature helps spread the virus throughout the body.

In dengue viremia, although virus has also been shown to circulate in the form of immune-complexes, a detailed study of the kinetics by which this occurs is still lacking. Thus, in general, a complete picture as to the nature of dengue viremia has not emerged primarily because it has been difficult to study this issue during the acute viremia period in human patients, since such patients are only seen following a febrile course at clinics where this issue is being studied. It is clearly possible that following the entry of the virus into the blood stream, the virus enters a permissive cell where it replicates in sufficient quantity to induce the febrile response, after which it can exist in several forms based on the level of viremia and host response to the viremia. It is therefore critical to establish a detailed analysis of these early events during the course of dengue virus infection, such as kinetics of viremia associated with each specific stage and the form the virus exists at during these specific stage, which may provide unique insights as to the physical properties of circulating dengue viral particles and may lead to the identification of unique targets for preventive vaccine development and the pathogenesis of DHF/DSS.

(iii) Conditioning of Neutralizing Antibody Assay in Dengue Virus Infection. In dengue endemic regions, a majority of the individuals are sera-positive for all four dengue serotypes by the time they reach adulthood. The intriguing phenomenon is that dengue viremia occurs even in the presence of significant levels of circulating antidengue virus antibody in these individuals, providing suggestive evidence that such antibodies are clearly nonneutralizing antibodies. However, it should be kept in mind that the measurement of neutralizing antibody levels to dengue virus has been predominantly derived on *in vitro* cultured dengue virus preparations. Thus the sources of viral stocks used for these studies may not be representative of wild-type virus and thus may influence the data obtained by the neutralization assays performed.

In reviewing the literature on this issue, three major sources of dengue virus stocks have been used for the virus neutralization studies. These include viral stocks cultured from C6/36 mosquito cells, Vero cells, and gradient-purified

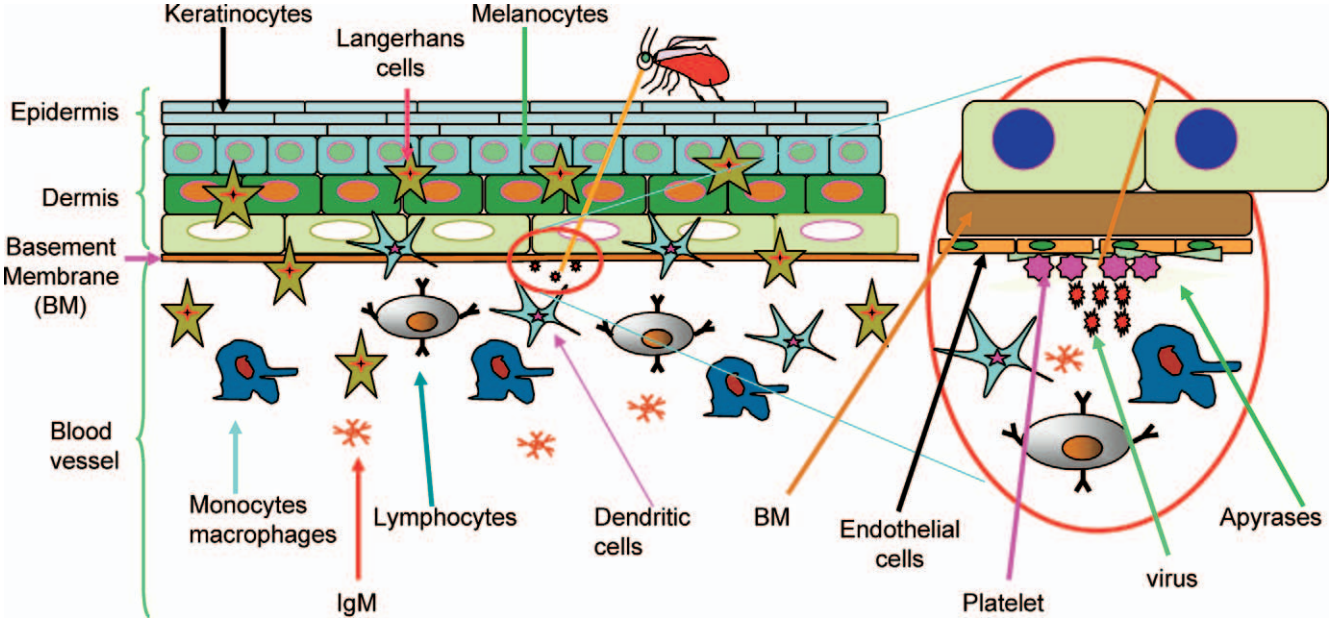

Figure 2. Innate immune encounter upon virus injection in the capillary vessel. There are several immune-related components circulated in the capillary, which is surrounded by multiple cell layers. The enlarged circle indicates the mosquito's probing site. Prior to the feeding process, mosquito saliva-containing factors (e.g., apyrases) and virus are released into the blood stream. A color figure is available in the online version.

virus preparations. The interpretation of the neutralizing antibody titer data on these stocks needs to be carefully evaluated. Thus as noted in the literature, differences in neutralization titers sometimes by twofold of magnitude have been reported even when using the same sera for the neutralization assay. In the era of evidence-based medicine, it is our opinion that the meaningful clinical relevance of the neutralizing assays can only be derived using primary virus isolates directly from dengue-infected patients, which to the best of our knowledge has not been investigated.

(iv) Concept of Vector Transmission. Dengue is transmitted by the bite of mosquito carrying the infectious dengue virus. The vector can serve as a biologic host also referred to as biologic transmission, which means that the virus is required to replicate within the vector before the virus can be transmitted to a new target host. In addition, the vector can serve as a transmitting vehicle, or so-called mechanical transmission, which means that the virus does not require replicating within the vector before it can be transmitted to a new target. While there are several reports on the biologic transmission of dengue virus in the mosquito vector, there is a paucity of information on the mechanical transmission of this virus. In endemic areas especially during an epidemic, mechanical transmission may play an important role in helping the spread of dengue virus. In both cases, the vector appears to directly inject the virus into capillary blood vessel (42).

Since dengue virus infection is initiated by injecting virus directly into the blood stream from the mosquito vector, it seems logical that initially at this stage, the virus predominantly encounters the host's innate immune system (Fig. 2). It thus seems reasonable to carry out more detailed studies of these initial events in efforts to identify at least some therapeutic strategies aimed at preventing these initial events of virus infection.

(v) Innate Immune System. The innate immune system is inborn in the host and is a universal and ancient form of host defense against infection. Thus, as the saying goes "phylogeny recapitulates ontogeny." It has been documented that the innate immune system exists without detectable forms of the adaptive immune system in more primitive phylogenetic species, which suggests that the innate immune system has had a longer evolutionary time to evolve. The innate immune system has been shown to consist of several components broadly ranging from skin barriers to cellular effector mechanisms and genome-encoded molecules, which act in concert to provide the host with an initial barrier against foreign organisms. The innate immune system thus includes natural antibody and factors involved in the maintenance of homeostasis (43–45). Key features of innate immunity are that it is not antigen-specific, and that it can respond immediately or within a few hours after encountering a novel intruder (43). The initial defense response by the body is to eliminate or temporarily contain the intruders and thereby to prevent or reduce the infection. The innate immune response to dengue infection, in particular the period between infection and appearance of clinical signs (preillness period, Fig. 3), is basically a subject that is to a large extent underexplored. We propose that two important components of the innate immune system, natural IgM antibody and platelets, are likely to play a critical role in the preillness period of dengue virus infection.

Natural IgM Antibody. Humoral immunity is mainly mediated by B cells, which produce different classes of antibodies, both natural and pathogen induced. Natural IgM

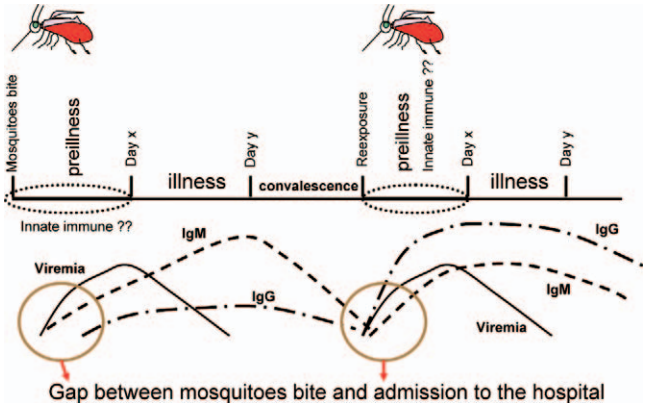

Figure 3. Preillness gaps. The innate immune factors between mosquito bites and the appearance of clinical signs are mysterious in dengue disease, either in primary infection or secondary infection. Viremia, IgM, and IgG are all readily detectable during the apparent clinical signs. The oval dotted lines indicate the parameters that are unknown between infection (mosquito bite) and the appearance of illness (day x, normally 2 to 5 days). The solid circles indicate the knowledge gaps in dengue virus infections, primary or secondary. Day y indicates viremia periods, which last from 5 to 7 days. Defervescence is the period that fever and viremia are recessed. A color figure is available in the online version.

antibodies are produced mainly by CD5+ B cells (B1 cells) and are a component of innate immunity (45–48). These nonspecific circulating natural antibodies can bind to pathogens, providing early host protection (49). Natural antibodies may facilitate antigen uptake, processing, and presentation by B lymphocytes via complement and Fc-receptors (50, 51). Interestingly, it has been suggested that B cells are the principal circulating mononuclear cells infected by dengue virus (52). Interactions between innate immune mechanisms and adaptive immune responses are now widely viewed as essential for a normal immune response (53–55).

Although in humans about 30% of the circulating antibodies are pentameric IgM molecules, a small amount of hexameric IgM, IgG, and IgA natural antibodies circulate as well (56). The physiologic roles of the natural IgM antibodies are not known. However, natural IgM antibodies have been shown to be involved in early recognition of external invaders and elimination of pathogens like bacteria and viruses (43, 57–65).

The multimeric structure makes IgM a strong complement activator; a single-bound IgM pentamer can trigger the classical pathway of complement activation and can lyse a red blood cell, while approximately a thousand IgG molecules are required to accomplish the same (66). Furthermore, the hexamer IgM, even though it circulates in smaller amounts, is 15 to 20 times more efficient in activating complement than the pentameric form of IgM (67–69). Circulating immune complexes (CIC) in sera of patients with DHF/DSS was first reported by Theofilopoulos *et al.* in 1976 (70). Dengue antigen can be detected in more than 50% of the CIC, and increased levels of platelet-associated IgM has been observed in DHF cases (71). Interestingly, IgM immune complex have been consistently found in the blood vessel walls of dermal papillae or cutaneous rashes of dengue patients (72–77). However, the origin and the structure of IgM in these immune complexes has never been characterized and described. Furthermore, the role of circulating IgM-immune complex in DHF/DSS is unknown. Perhaps, immune complexes that attach to the surface of platelets may enhance platelet destruction by the reticulo-endothelial system in the liver and the spleen, resulting in thrombocytopenia during the shock phase of disease. Therefore, the levels of IgM, notably natural IgM that has specificity for dengue viruses, in an individual may have an impact on the outcome of dengue infection.

Platelets. Platelets are an essential element in hemostasis (78). In recent studies, platelets have been viewed as an integral part of the innate immune system and can be potent effectors of the innate immune response (79, 80). In addition, a functional CD154 (CD40L), a molecule shown to be critical for the enhancement of antigen presentation and augmentation of the adaptive immune response, has recently been shown to be expressed on the surface of platelets (81, 82). This further supports a role of platelets in modulating the immune response and inflammation (80, 83).

Platelets are anucleated, composed of a concentric megakaryocyte membrane, cytoplasm, granules, and organelles. Platelets are produced within the bone marrow and the cells from which platelets originate are called megakaryocytes (84). Platelets circulate throughout blood vessels and function to monitor the integrity of the vascular system. There are two forms of platelets, a resting form circulating as discs and an active form circulating as filopodia. Structurally, these forms have different arrangements of internal microtubules and actin cytoskeleton (84).

When the lining of a blood vessel is traumatized, platelets are stimulated to go to the site of the injury, where they form a plug to reduce the loss of blood. All platelet functional responses must be tightly regulated to ensure that the formation of a blood clot is of sufficient size to seal off the damaged area, while not disrupting blood flow to vital organs by causing vessel occlusion (85, 86). Thus, impairment of platelet function can increase risk of vascular fragility leading to hemorrhage. This may be an important mechanism of plasma leakage in severe dengue disease (DHF/DSS) (18).

One of the key clinical manifestations in dengue disease is thrombocytopenia, otherwise known as low platelet counts (17). This arises from both decreased production (87, 88) and increased destruction of platelets (77, 89–92). The degree of thrombocytopenia appears well-correlated not only with the clinical severity of DHF, but also with the activation of the complement system (93, 94).

During dengue virus infection, platelets may provide a wonderful shield for the virus from exposure and binding to neutralizing preexisting antibody. Interestingly, there are a few reports suggesting that dengue virus may associate with platelets, directly or indirectly through antibody (75, 77, 91, 92, 95, 96). Assuming this is the case, we can envision that the virus-platelets-antibody complexes may enhance the

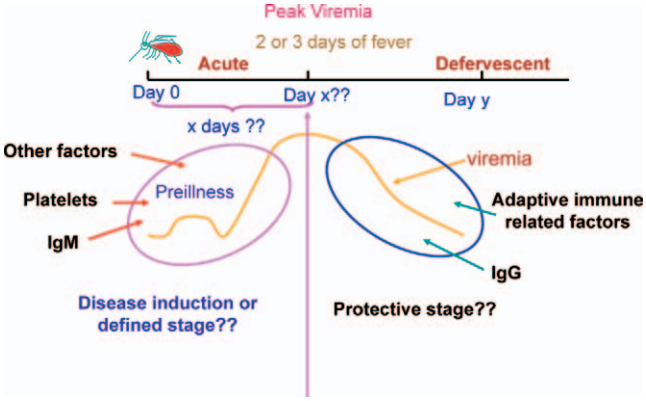

Figure 4. Role of innate immune parameters in DHF/DSS. The proposed alternate hypothesis on DHF/DSS can potentially divide into two stages, disease induction or defined stage and protective stage. Innate immune-related parameters, such as other factors (cytokines, etc.), natural IgM, or platelets, may directly or indirectly contribute to define the development of DHF/DSS during the course of early dengue virus infection (purple circle, preillness). While in the protective stage, the periods in which fever and viral load are recessed, DHF/DSS may attribute to antigen-induced factors, such as IgG and adaptive immune response (blue circle). The purple arrow indicates the time in which, at or around the peak viremia stage, patients reported to the clinics or hospitals, normally after 2 or 3 days of fever. The orange curve and arrow indicate the typical kinetic of viremia. x and y are defined as in Figure 3. A color figure is available in the online version.

phagocytosis or be engulfed by macrophages or monocytes via Fcγ receptor as suggested by others (10). In addition, relatively recently dengue virus RNA has been detected using reverse transcription polymerase chain reaction techniques in RNA purified from platelets from dengue infected patients (71). The interesting point in these observations or reports is that the thrombocytopenia seen in DHF/DSS patients may not only be caused by the destruction of platelets by the virus itself (direct cytotoxicity), but may also be caused by the destruction of platelets following the binding of dengue-specific antibodies to the virus-infected platelets (immune-mediated toxicity) (97. It is also possible that platelets can serve as a reservoir for dengue virus replication; however, this issue is a subject of further investigation.

Alternate Explanation on DHF/DSS. Our take-home message in this short review on alternate hypothesis is summarized in Figure 4. Under this scheme, there is a potentially important role of naturally occurring IgM antibodies that may play an important role in the early acute dengue viral infection, which may set the stage for the clinical course of the disease. In addition, a role for platelets as the source of primary infection and/or as carriers of the virus and the subsequent innate immune response against these virus infected platelets may play a pivotal role in the induction of DHF/DSS. Our laboratory is currently focused on addressing this issue in a formal study.

The dynamic and complexity of dengue disease immediately prompts us to realize that the alternative hypothesis in this short review will not be sufficient to cover all the various other hypothesis that have been forwarded in the extensive amount of literature. Readers, therefore, should refer to recent comprehensive reviews on dengue pathogenesis (44, 98–105).

Conclusions

An alternate hypothesis on the pathogenesis of DHF/DSS is presented in this short review. It is the objective of the authors to draw scientific attention to these new concepts, which we hope contribute to providing a much more complete picture on dengue pathogenesis. Our aim is to encourage more detailed studies of the acute viremia period, which we believe will not only provide useful information of the mechanisms associated with DHF/DSS, but will also assist in the formulation of effective candidate vaccines or drug development.

We thank Professor Aftab A. Ansari, Department of Pathology and Laboratory Medicine, School of Medicine, Emory University, for his helpful discussions and knowledgeable suggestions in the writing of this manuscript.

1. Guzman MG, Kouri G. Dengue: an update. Lancet Infect Dis 2: 33–42, 2002.
2. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 11:480–496, 1998.
3. Chambers TJ, Hahn CS, Galler R, Rice CM. Flavivirus genome organization, expression, and replication. Annu Rev Microbiol 44: 649–688, 1990.
4. Clark G, Gubler D, Rigau J. Dengue fever. CDC traveler's information on dengue fever. Atlanta, GA: Centers for Disease Control. Available at: http://cdc.gov/travel/diseases/dengue.htm.
5. Guzman M, Kouri G, Diaz M, Llop A, Vazquez S, Gonzalez D, Castro O, Alvarez A, Fuentes O, Montada D, Padmanabha H, Sierra B, Perez A, Rosario D, Pupo M, Diaz C, Sanchez L. Dengue, one of the great emerging health challenges of the 21st century. Expert Rev Vaccines 3:511–520, 2004.
6. National Institute of Allergy and Infectious Diseases. Dengue Fever. Baltimore, MD: National Institutes of Health, 2005.
7. World Health Organization. Prevention and control of dengue and dengue haemorrhagic fever. World Health Organization regional publication, SEARO. Geneva: World Health Organization, 1999.
8. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg 38:172–180, 1988.
9. Guzman MG. Global voices of science. Deciphering dengue: the Cuban experience. Science 309:1495–1497, 2005.
10. Halstead SB. Pathogenesis of dengue: challenges to molecular biology. Science 239:476–481, 1988.
11. Halstead SB, O'Rourke EJ. Antibody-enhanced dengue virus infection in primate leukocytes. Nature 265:739–741, 1977.
12. Rosen L. The Emperor's New Clothes revisited, or reflections on the pathogenesis of dengue hemorrhagic fever. Am J Trop Med Hyg 26: 337–343, 1977.
13. Kurane I, Ennis FA. Immunopathogenesis of dengue virus infections. In: Gubler DJ, Kuno G, Eds. Dengue and Dengue Hemorrhagic Fever. Wallingford: CAB International, pp273–290, 1997.
14. Spaulding AC, Kurane I, Ennis FA, Rothman AL. Analysis of murine CD8(+) T-cell clones specific for the Dengue virus NS3 protein: flavivirus cross-reactivity and influence of infecting serotype. J Virol 73:398–403, 1999.
15. Mathew A, Kurane I, Green S, Stephens HA, Vaughn DW, Kalayanarooj S, Suntayakorn S, Chandanayingyong D, Ennis FA, Rothman AL. Predominance of HLA-restricted cytotoxic T-lymphocyte responses to serotype-cross-reactive epitopes on nonstructural

proteins following natural secondary dengue virus infection. J Virol 72:3999–4004, 1998.
16. Gubler DJ. Dengue/dengue haemorrhagic fever: history and current status. Novartis Found Symp 277:3–16; discussion 16–22, 71–73, 251–253, 2006.
17. Halstead SB. Epidemiology of dengue and dengue haemorrhagic fever. In: Gubler DJ, Kuno, G, Eds. Dengue and Dengue Haemorrhagic Fever. Wallingford: CAB International, pp23–44, 1997.
18. World Health Organization. Dengue and dengue haemorrhagic fever. Chapter 6. In: WHO Report on global surveillance of epidemic prone infectious diseases, Geneva: World Health Organization, 2001.
19. Chen JP, Cosgriff TM. Hemorrhagic fever virus-induced changes in hemostasis and vascular biology. Blood Coagul Fibrinolysis 11: 461–483, 2000.
20. Lei HY, Yeh TM, Liu HS, Lin YS, Chen SH, Liu CC. Immunopathogenesis of dengue virus infection. J Biomed Sci 8:377–388, 2001.
21. Guzman MG, Kouri G, Bravo J, Valdes L, Vazquez S, Halstead SB. Effect of age on outcome of secondary dengue 2 infections. Int J Infect Dis 6:118–124, 2002.
22. Iyngkaran N, Yadav M, Sinniah M. Augmented inflammatory cytokines in primary dengue infection progressing to shock. Singapore Med J 36:218–221, 1995.
23. Green S, Vaughn DW, Kalayanarooj S, Nimmannitya S, Suntayakorn S, Nisalak A, Lew R, Innis BL, Kurane I, Rothman AL, Ennis FA. Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. J Infect Dis 179:755–762, 1999.
24. Gagnon SJ, Mori M, Kurane I, Green S, Vaughn DW, Kalayanarooj S, Suntayakorn S, Ennis FA, Rothman AL. Cytokine gene expression and protein production in peripheral blood mononuclear cells of children with acute dengue virus infections. J Med Virol 67:41–46, 2002.
25. Liu CC, Huang KJ, Lin YS, Yeh TM, Liu HS, Lei HY. Transient CD4/CD8 ratio inversion and aberrant immune activation during dengue virus infection. J Med Virol 68:241–252, 2002.
26. Suharti C, van Gorp EC, Dolmans WM, Setiati TE, Hack CE, Djokomoeljanto R, van der Meer JW. Cytokine patterns during dengue shock syndrome. Eur Cytokine Netw 14:172–177, 2003.
27. Anderson R, Wang S, Osiowy C, Issekutz AC. Activation of endothelial cells via antibody-enhanced dengue virus infection of peripheral blood monocytes. J Virol 71:4226–4232, 1997.
28. Carr JM, Hocking H, Bunting K, Wright PJ, Davidson A, Gamble J, Burrell CJ, Li P. Supernatants from dengue virus type-2 infected macrophages induce permeability changes in endothelial cell monolayers. J Med Virol 69:521–528, 2003.
29. Lin CF, Lei HY, Shiau AL, Liu CC, Liu HS, Yeh TM, Chen SH, Lin YS. Antibodies from dengue patient sera cross-react with endothelial cells and induce damage. J Med Virol 69:82–90, 2003.
30. Pang T, Cardosa MJ, Guzman MG. Of cascades and perfect storms: the immunopathogenesis of dengue haemorrhagic fever-dengue shock syndrome (DHF/DSS). Immunol Cell Biol 85:43–45, 2007.
31. Sierra Bde L, Kouri G, Guzman MG. Race: a risk factor for dengue hemorrhagic fever. Arch Virol 152:533–542, 2007.
32. Zulkarnain E, Hotta S, Takegami T. Molecular comparison of dengue type 1 Mochizuki strain virus and other selected viruses concerning nucleotide and amino acid sequences of genomic RNA: a consideration of viral epidemiology and variation. Microbiol Immunol 38: 581–585, 1994.
33. Diamond MS, Edgil D, Roberts TG, Lu B, Harris E. Infection of human cells by dengue virus is modulated by different cell types and viral strains. J Virol 74:7814–7823, 2000.
34. Gubler DJ, Suharyono W, Tan R, Abidin M, Sie A. Viraemia in patients with naturally acquired dengue infection. Bull World Health Organ 59:623–630, 1981.
35. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 181:2–9, 2000.
36. Thisyakorn U, Nimmannitya S. Nutritional status of children with dengue hemorrhagic fever. Clin Infect Dis 16:295–297, 1993.
37. Leon LR. Invited review: cytokine regulation of fever: studies using gene knockout mice. J Appl Physiol 92:2648–2655, 2002.
38. Paranjape S, Patil BR, Kadam VD. Characterization of porcine stable kidney cell line adapted to hyperthermic temperature. In Vitro Cell Dev Biol Anim 39:193–195, 2003.
39. Paranjape SP, Kadam VD, Deolankar RP. Increased yields of Japanese encephalitis virus in heat shocked cell cultures. Acta Virol 38:333–337, 1994.
40. Kuno G, Oliver A. Maintaining mosquito cell lines at high temperatures: effects on the replication of flaviviruses. In Vitro Cell Dev Biol 25:193–196, 1989.
41. Mandel B. Neutralization of animal viruses. Adv Virus Res 23:205–268, 1978.
42. Gordon RM, Lumsden WHR. A study of the behaviour of the mouthparts of mosquitoes when taking up blood from living tissues; together with some observations on the ingestion of microfilariae. Ann Trop Med Parasit 33:259–278, 1939.
43. Janeway CA, Jr., Medzhitov R. Innate immune recognition. Annu Rev Immunol 20:197–216, 2002.
44. Navarro-Sanchez E, Despres P, Cedillo-Barron L. Innate immune responses to dengue virus. Arch Med Res 36:425–435, 2005.
45. Boes M. Role of natural and immune IgM antibodies in immune responses. Mol Immunol 37:1141–1149, 2000.
46. Casali P, Notkins AL. CD5+ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. Immunol Today 10:364–368, 1989.
47. Vollmers HP, O'Connor R, Muller J, Kirchner T, Muller-Hermelink HK. SC-1, a functional human monoclonal antibody against autologous stomach carcinoma cells. Cancer Res 49:2471–2476, 1989.
48. Kantor AB, Herzenberg LA. Origin of murine B cell lineages. Annu Rev Immunol 11:501–538, 1993.
49. Gobet R, Cerny A, Ruedi E, Hengartner H, Zinkernagel RM. The role of antibodies in natural and acquired resistance of mice to vesicular stomatitis virus. Exp Cell Biol 56:175–180, 1988.
50. Carroll MC. The role of complement and complement receptors in induction and regulation of immunity. Annu Rev Immunol 16: 545–568, 1998.
51. Thornton BP, Vetvicka V, Ross GD. Natural antibody and complement-mediated antigen processing and presentation by B lymphocytes. J Immunol 152:1727–1737, 1994.
52. King AD, Nisalak A, Kalayanrooj S, Myint KS, Pattanapanyasat K, Nimmannitya S, Innis BL. B cells are the principal circulating mononuclear cells infected by dengue virus. Southeast Asian J Trop Med Public Health 30:718–728, 1999.
53. Fearon DT, Locksley RM. The instructive role of innate immunity in the acquired immune response. Science 272:50–53, 1996.
54. Medzhitov R, Janeway CA, Jr. Innate immunity: the virtues of a nonclonal system of recognition. Cell 91:295–298, 1997.
55. Carroll MC, Prodeus AP. Linkages of innate and adaptive immunity. Curr Opin Immunol 10:36–40, 1998.
56. Avrameas S. Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton'. Immunol Today 12:154–159, 1991.
57. Janeway CA. Autoimmune disease: immunotherapy by peptides? Nature 341:482–483, 1989.
58. Ochsenbein AF, Pinschewer DD, Odermatt B, Carroll MC, Hengartner H, Zinkernagel RM. Protective T cell-independent antiviral antibody responses are dependent on complement. J Exp Med 190: 1165–1174, 1999.
59. Ulvestad E, Wilfred LL, Kristoffersen EK. Measurement of IgM rheumatoid factor by ELISA. Scand J Rheumatol 30:366, 2001.
60. Brandlein S, Vollmers HP. Natural IgM antibodies, the ignored weapons in tumour immunity. Histol Histopathol 19:897–905, 2004.

61. Boes M, Prodeus AP, Schmidt T, Carroll MC, Chen J. A critical role of natural immunoglobulin M in immediate defense against systemic bacterial infection. J Exp Med 188:2381–2386, 1998.
62. Ben-Aissa-Fennira F, Ben Ammar-El Gaaied A, Bouguerra A, Dellagi K. IgM antibodies to P1 cytoadhesin of Mycoplasma pneumoniae are part of the natural antibody repertoire expressed early in life. Immunol Lett 63:59–62, 1998.
63. Baumgarth N, Herman OC, Jager GC, Brown LE, Herzenberg LA, Chen J. B-1 and B-2 cell-derived immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection. J Exp Med 192:271–280, 2000.
64. Ulvestad E, Kanestrom A, Sonsteby LJ, Jureen R, Omland T, Edvardsen B, Lundervik J, Kristoffersen E, van Dam AP. Diagnostic and biologic significance of anti-p41 IgM antibodies against Borrelia burgdorferi. Scand J Immunol 53:416–421, 2001.
65. Rodman TC, Lutton JD, Jiang S, Al-Kouatly HB, Winston R. Circulating natural IgM antibodies and their corresponding human cord blood cell-derived Mabs specifically combat the Tat protein of HIV. Exp Hematol 29:1004–1009, 2001.
66. Cooper NR, Nemerow GR. Complement effector mechanisms in health and disease. J Invest Dermatol 85:39s–46s, 1985.
67. Davis AC, Roux KH, Shulman MJ. On the structure of polymeric IgM. Eur J Immunol 18:1001–1008, 1988.
68. Randall TD, King LB, Corley RB. The biological effects of IgM hexamer formation. Eur J Immunol 20:1971–1979, 1990.
69. Wiersma EJ, Collins C, Fazel S, Shulman MJ. Structural and functional analysis of J chain-deficient IgM. J Immunol 160:5979–5989, 1998.
70. Theofilopoulos AN, Brandt WE, Russell PK, Dixon FT. Replication of dengue-2 virus in cultured human lymphoblastoid cells and subpopulations of human peripheral leukocytes. J Immunol 117:953–961, 1976.
71. Saito M, Oishi K, Inoue S, Dimaano EM, Alera MT, Robles AM, Estrella BD, Jr., Kumatori A, Moji K, Alonzo MT, Buerano CC, Matias RR, Morita K, Natividad FF, Nagatake T. Association of increased platelet-associated immunoglobulins with thrombocytopenia and the severity of disease in secondary dengue virus infections. Clin Exp Immunol 138:299–303, 2004.
72. Halstead SB. Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypotheses and discussion. Yale J Biol Med 42:350–362, 1970.
73. Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J Exp Med 146:201–217, 1977.
74. Boonpucknavig S, Boonpucknavig V, Bhamarapravati N, Nimmannitya S. Immunofluorescence study of skin rash in patients with dengue hemorrhagic fever. Arch Pathol Lab Med 103:463–466, 1979.
75. Boonpucknavig S, Vuttiviroj O, Bunnag C, Bhamarapravati N, Nimmanitya S. Demonstration of dengue antibody complexes on the surface of platelets from patients with dengue hemorrhagic fever. Am J Trop Med Hyg 28:881–884, 1979.
76. Ruangjirachuporn W, Boonpucknavig S, Nimmanitya S. Circulating immune complexes in serum from patients with dengue haemorrhagic fever. Clin Exp Immunol 36:46–53, 1979.
77. Phanichyakarn P, Pongpanich B, Israngkura PB, Dhanamitta S, Valyasevi A. Studies on Dengue hemorrhagic fever. III. Serum complement (C3) and platelet studies. J Med Assoc Thai 60:301–306, 1977.
78. George JN. Platelets. Lancet 355:1531–1539, 2000.
79. Klinger MH. Platelets and inflammation. Anat Embryol (Berl) 196: 1–11, 1997.
80. Weyrich AS, Zimmerman GA. Platelets: signaling cells in the immune continuum. Trends Immunol 25:489–495, 2004.
81. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, Kroczek RA. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 391:591–594, 1998.
82. Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, Stein CS, Nieswandt B, Wang Y, Davidson BL, Ratliff TL. Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. Immunity 19: 9–19, 2003.
83. Elzey BD, Sprague DL, Ratliff TL. The emerging role of platelets in adaptive immunity. Cell Immunol 238:1–9, 2005.
84. Hartwig JH. The platelet: form and function. Semin Hematol 43: S94–S100, 2006.
85. Andrews RK, Berndt MC. Platelet physiology and thrombosis. Thromb Res 114:447–453, 2004.
86. Rendu F, Brohard-Bohn B. The platelet release reaction: granules' constituents, secretion and functions. Platelets 12:261–273, 2001.
87. La Russa VF, Innis BL. Mechanisms of dengue virus-induced bone marrow suppression. Baillieres Clin Haematol 8:249–270, 1995.
88. Rosenfeld SJ, Young NS. Viruses and bone marrow failure. Blood Rev 5:71–77, 1991.
89. Mitrakul C, Poshyachinda M, Futrakul P, Sangkawibha N, Ahandrik S. Hemostatic and platelet kinetic studies in dengue hemorrhagic fever. Am J Trop Med Hyg 26:975–984, 1977.
90. Malasit P. Complement and dengue haemorrhagic fever/shock syndrome. Southeast Asian J Trop Med Public Health 18:316–320, 1987.
91. Funahara Y, Ogawa K, Fujita N, Okuno Y. Three possible triggers to induce thrombocytopenia in dengue virus infection. Southeast Asian J Trop Med Public Health 18:351–355, 1987.
92. Wang S, He R, Patarapotikul J, Innis BL, Anderson R. Antibody-enhanced binding of dengue-2 virus to human platelets. Virology 213: 254–257, 1995.
93. Pathogenetic mechanisms in dengue haemorrhagic fever: report of an international collaborative study. Bull World Health Organ 48:117–133, 1973.
94. Edelman R, Nimmannitya S, Colman RW, Talamo RC, Top FH, Jr. Evaluation of the plasma kinin system in dengue hemorrhagic fever. J Lab Clin Med 86:410–421, 1975.
95. Lin CF, Lei HY, Liu CC, Liu HS, Yeh TM, Wang ST, Yang TI, Sheu FC, Kuo CF, Lin YS. Generation of IgM anti-platelet autoantibody in dengue patients. J Med Virol 63:143–149, 2001.
96. Oishi K, Inoue S, Cinco MT, Dimaano EM, Alera MT, Alfon JA, Abanes F, Cruz DJ, Matias RR, Matsuura H, Hasebe F, Tanimura S, Kumatori A, Morita K, Natividad FF, Nagatake T. Correlation between increased platelet-associated IgG and thrombocytopenia in secondary dengue virus infections. J Med Virol 71:259–264, 2003.
97. Oishi K, Saito M, Mapua CA, Natividad FF. Dengue illness: clinical features and pathogenesis. J Infect Chemother 13:125–133, 2007.
98. Clyde K, Kyle JL, Harris E. Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis. J Virol 80:11418–11431, 2006.
99. Chaturvedi UC, Nagar R, Shrivastava R. Macrophage and dengue virus: friend or foe? Indian J Med Res 124:23–40, 2006.
100. Chaturvedi UC, Shrivastava R, Tripathi RK, Nagar R. Dengue virus-specific suppressor T cells: current perspectives. FEMS Immunol Med Microbiol, 50:285–299, 2007.
101. Lin CF, Wan SW, Cheng HJ, Lei HY, Lin YS. Autoimmune pathogenesis in dengue virus infection. Viral Immunol 19:127–132, 2006.
102. Screaton G, Mongkolsapaya J. T cell responses and dengue haemorrhagic fever. Novartis Found Symp 277:164–171; discussion 171–166, 251–253, 2006.
103. Green S, Rothman A. Immunopathological mechanisms in dengue and dengue hemorrhagic fever. Curr Opin Infect Dis 19:429–436, 2006.
104. Fink J, Gu F, Vasudevan SG. Role of T cells, cytokines and antibody in dengue fever and dengue haemorrhagic fever. Rev Med Virol 16: 263–275, 2006.
105. Schroder JM, Reich K, Kabashima K, Liu FT, Romani N, Metz M, Kerstan A, Lee PH, Loser K, Schon MP, Maurer M, Stoitzner P, Beissert S, Tokura Y, Gallo RL. Who is really in control of skin immunity under physiological circumstances-lymphocytes, dendritic cells or keratinocytes? Exp Dermatol 15:913–929, 2006.