Platelets in Dengue Infection

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Haematology

Platelets in dengue infection

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Dengue has established itself as one of the world's most common mosquito-borne viral diseases. Although it prevails in tropical areas, sustained transmission of dengue has recently occurred in Florida. Dengue viruses can induce a spectrum of symptoms and, in severe cases, mortality in approximately 1–5% of infected individuals. A hallmark of dengue infection is thrombocytopenia that associates with abnormal platelet function, which is the focus of this review.

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Introduction

Dengue is an endemic disease that occurs throughout the world; over 2.5 billion people live in high-risk tropical or subtropical transmission areas [1]. It is the most common human arbovirus infection and is responsible for thousands of deaths every year. Estimates suggest that 50 million dengue infections occur annually with up to 500,000 cases of dengue hemorrhagic fever (DHF) and at least 22,000 reported deaths [2].

The dengue virus (DV) induces a spectrum of clinical manifestations that range from a self-limited fever to a severe form (DHF), which may progress to shock and death. Although not fully elucidated, recent evidence indicates that severe DV infections increase vascular permeability that leads to decreased intravascular fluid volume and consequent hemoconcentration and hypotension in infected patients. Another feature of DV infection is thrombocytopenia, which is common in both mild and severe diseases (Table 1) [3–7].

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Molecular mechanisms of thrombocytopenia in dengue infection

DV is transmitted by mosquitoes of the genus *Aedes*, usually *Aedes aegypti*, a synanthropic species associated with human habitation that is expanding rapidly in many areas of the world [8]. The DV belongs to the family Flaviviridae and comprises four (DV-1–DV-4) antigenically distinct serotypes [9]. The DV is composed of a nucleocapsid that is surrounded by a lipid membrane and covered by glycoproteins (Fig. 1). Its genome consists of a positive-sense, single-stranded RNA that encodes a polyprotein precursor of viral proteins [10]. This precursor is cleaved by host and viral proteases, which generates three structural (C, capsid; prM, premembrane; and E, envelope) and seven nonstructural (NS1, NS2a, NS2b, NS3, NS4A, NS4B and NS5) proteins [11]. The structural proteins are incorporated into the mature infective virion, while the nonstructural proteins are involved in the replication and assembly of the virus [8–11].

DV has been isolated from polymorphonuclear leukocytes, monocyte/macrophages, dendritic cells and others [12]. It has also been detected in megakaryocyte progenitors and circulating platelets [13–15]. These findings suggest that DV may induce thrombocytopenia via direct interactions with megakaryocytes and platelets. DV has also been shown to reduce circulating platelet counts independent of virus attachment or entry into platelets or their precursors. Thus, two mechanisms

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Table 1. Thrombocytopenia (<100,000 platelets/mm^3) during DENV infection in hospital-based studies.

Study location	DF	DHF	DSS	Authors
Percent of thrombocytopenic patients				
Children's Hospital, Bangkok	50.2%	93.8%	92.1%	Kalayanarooj *et al.* [4]
Tropical Medicine Foundation of Amazonas, Brazil	21.1%[a]	45.5%[a]	NR	Mourao *et al.* [5]
Hospitals in Niterói (Rio de Janeiro), Brazil	63%	72%	NR	Bozza *et al.* [6][b]
Mean platelet count (×1000/mm^3)				
Children's Hospital, Bangkok	123.599	63.855	53.452	Kalayanarooj *et al.* [4]
San Lazaro Hospital, Manila	60.0 (±24.3)	39.3 (±30.8)	NR	Honda *et al.* [7]

Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome.
[a] Percent of patients with severe thrombocytopenia (<50,000 platelets/mm^3).
[b] In this paper patients were classified as classic dengue or severe dengue.

are probably involved in dengue-induced thrombocytopenia: impaired thrombopoiesis and peripheral platelet destruction (Fig. 2).

Impaired thrombopoiesis

Marrow suppression within 2–4 days of DV infection can contribute to thrombocytopenia (Fig. 3). Viral RNA has been isolated from bone marrow specimens of infected individuals, suggesting that dengue targets the marrow and hematopoietic system [16]. Bone marrow studies also reveal diminished megakaryopoiesis during the onset of dengue infection and clinical recovery is associated with normal megakaryocyte topography and platelet counts [17].

Suppression of megakaryopoiesis occurs either directly, due to infection and suppression of hematopoietic progenitor cells or indirectly, via impairment of stromal cells that function by altering the repertoire of cytokines in the bone marrow microenvironment. In regard to direct effects, Nakao *et al.* [13] demonstrated that DV-4 propagates in human bone marrow progenitors *in vitro* and alters their proliferative capacity. DV infection suppresses proliferation of human cord blood progenitors and DV-2 inhibits the differentiation of CD34$^+$ progenitors into megakaryocytes, presumably by inducing apoptosis in infected cells [18,19]. Together, these data support that DV is able to directly infect hematopoietic progenitors and suppress megakaryopoiesis and thrombopoiesis.

DV can also infect stromal cells, which in turn suppresses hematopoiesis. Rothwell *et al.* [20] infected long-term marrow cultures with DV-2 and characterized the viral antigen-positive cells. This investigation demonstrated two types of stromal cells that were positive for viral antigens: adventitial reticular cells and bone marrow dendritic cells. Moreover, DV infected stroma did not support colony-forming cells either in coculture or as a feeder layer separated from highly purified CD34$^+$ progenitor cells. The cytokine profile was different among infected and uninfected stromal cells, suggesting that altered cytokine production by infected stroma is the most probable mechanism of marrow suppression during DV infection.

The *in vitro* findings described above and the hematological findings of leukopenia in conjunction with thrombocytopenia in dengue patients are used as argument in favor of dengue globally suppressing bone marrow hematopoiesis [17]. However, emerging evidence indicates that dengue infection also has extramedullary effects on circulating platelets.

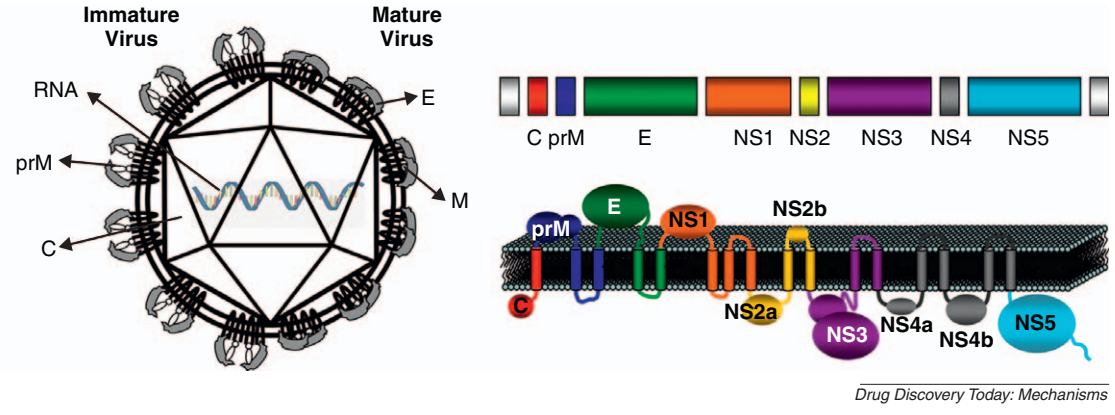

Figure 1. The dengue virus structure and genome. **(a)** Schematic representation of structural proteins in immature and mature dengue viral particle. **(b)** Schematic representation of the flavivirus genome organization and expression: structural and nonstructural protein coding regions and topology of the polyprotein in the membrane of endoplasmic reticulum.

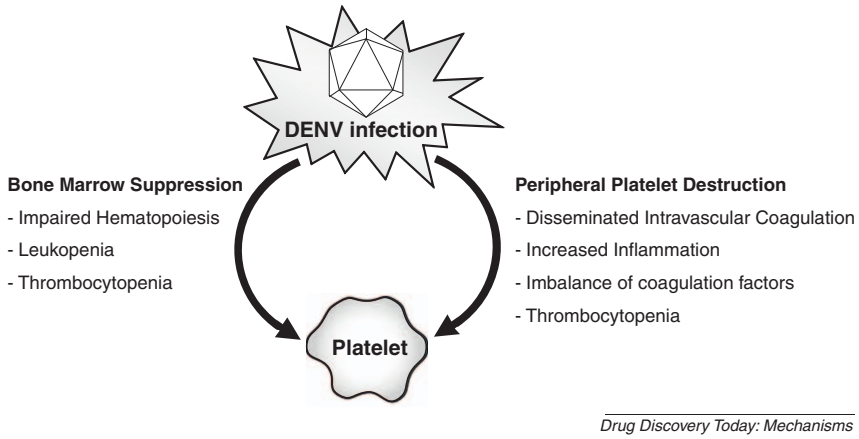

Figure 2. Schematic of bone marrow suppression and peripheral platelet destruction elicited by the dengue virus (DV). Schematic representation of two major mechanisms involved in dengue-induced thrombocytopenia: bone marrow suppression and peripheral platelet destruction. Text boxes describe findings that support each mechanism.

Increased peripheral destruction

Autoimmune-induced platelet activation and clearance

Several groups have put forth the autoimmune hypothesis, which postulates that host-generated anti-DV antibodies crossreact with platelets and facilitate their clearance [21]. In support of this concept, serum from dengue patients can bind platelets and higher levels of antiplatelet IgM are observed in severe DV infections when compared to classical dengue fever [22]. Moreover, dengue patient serum or rabbit antinonstructural protein-1 (NS1) induce complement-mediated lysis in platelets [22,23], which may contribute to the loss of circulating platelets during dengue illness. Autoantibodies directed against NS1 target human platelets and fibrinogen and induce thrombocytopenia in mice [23,24]. A molecular mimicry mechanism has been proposed in which the C-terminal region of NS1 shows sequence homology with integrins on the surface of platelets [25]. Similarly, different regions of the C, prM and E proteins of dengue display sequence homology with coagulatory molecules such as thrombin, plasminogen and tissue plasminogen activator [25].

In clinical settings, increased levels of platelet-associated immunoglobulin (PAIgM or PAIgG) and phagocytosis of platelets by macrophages correlates with thrombocytopenia during the acute phase of secondary dengue infection [7,15]. Similarly, anti-NS1 autoantibodies or pooled sera from dengue patients enhance the engagement of immunoglobulin-opsonized platelets by macrophages [23]. Nevertheless, administration of intravenous immunoglobulin, which is commonly used to treat patients with idiopathic thrombocytopenic purpura, does not increase platelet counts in dengue-infected patients [26]. This suggests that dengue-induced thrombocytopenia may not rely on Fc receptor mediated phagocytosis of platelets.

Platelet–leukocyte and platelet–endothelial cell interactions

Similar to platelets, antibodies directed against DV NS1 cross-react and activate endothelial cells [27]. Additionally, productive DV infection and activation of endothelial cells has also been reported [28,29]. Endothelial cells infected with DV display high expression of E-selectin and support the adherence of platelets [30].

Platelets that adhere to dengue-infected endothelial cells express surface P-selectin [31]. P-selectin expressing platelets are known to interact with leukocytes and DV-induced hemorrhage elicits platelet–monocyte and platelet–neutrophil aggregates in a primate model system [12]. Platelet–monocyte aggregates are also frequently observed in humans infected with dengue [32]. Increased interactions of platelets with leukocytes and endothelium are probably contributors to the pathogenesis of dengue disease, including thrombocytopenia.

Platelet dysfunction

There are a few studies examining platelet function in dengue disease. Among these, it has been shown that dengue serum abnormally activates platelets and inhibits platelet aggregation [22,23]. One mechanism for attenuated platelet aggregation involves recognition of protein disulfide isomerase (PDI) on the surface of platelets by anti-DV NS1 antibodies [33]. Specifically, anti-DV NS1 antibodies from hyperimmunized mouse sera inhibit PDI activity and platelet aggregation, a response that is alleviated when the antibodies are preabsorbed by PDI. Inhibition of platelet aggregation is induced by antibodies generated against full-length NS1, but not antibodies lacking the C-terminal region of NS1, which exhibit higher platelet binding and inactivate integrin $\alpha_{IIb}\beta_3$ [34]. Decreased platelet aggregation has also been attributed to increased L-arginine transport and nitric oxide generation in platelets from dengue patients [35]. Whether altered platelet

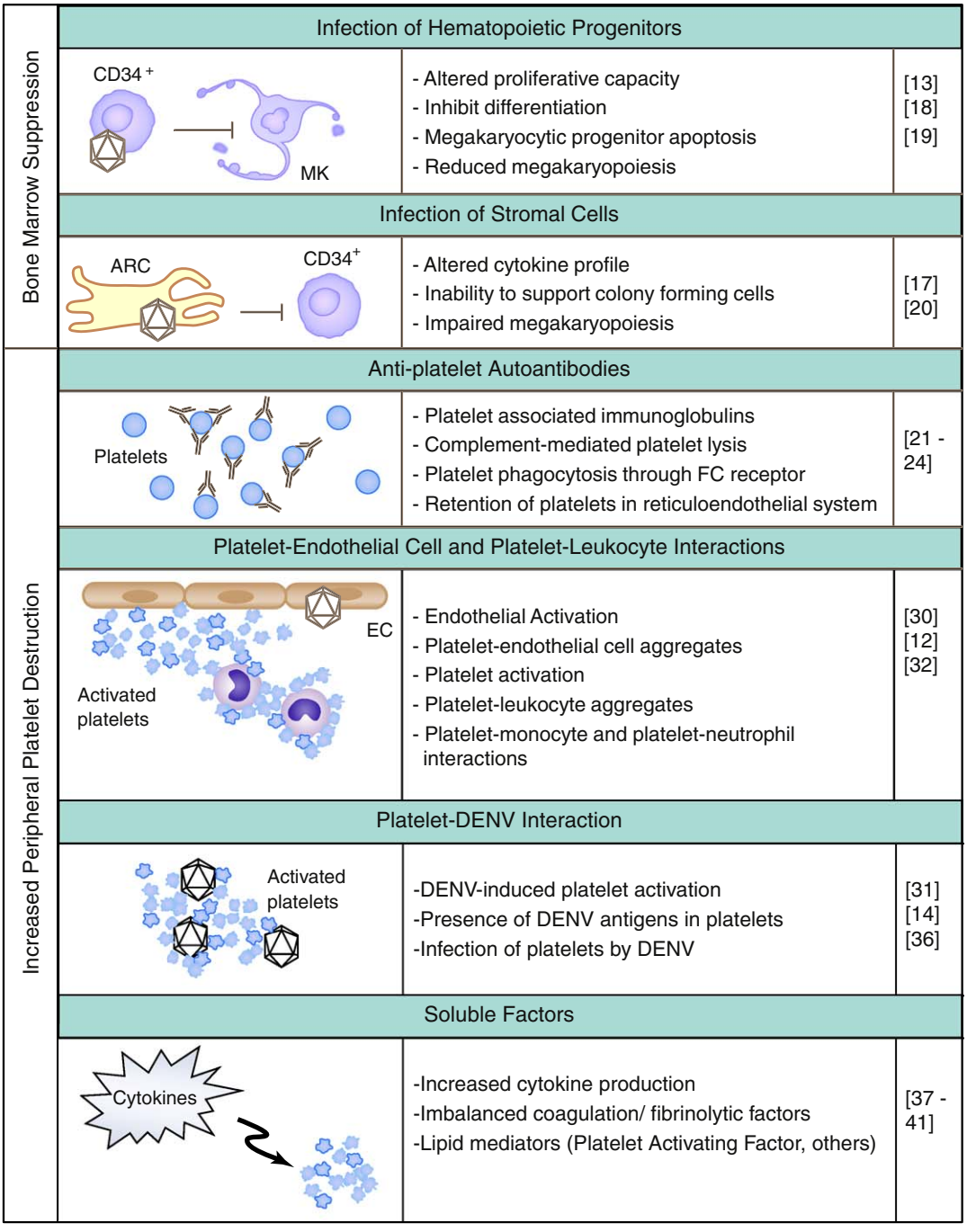

Figure 3. Overview of molecular mechanisms that contribute to dengue-induced thrombocytopenia.

function occurs *in vivo* and contributes to dengue-induced thrombocytopenia is not known.

Direct infection

Recent studies indicate that DV directly interacts and activates platelets. DV induces morphological changes in normal platelets typical of activation, including the presence of filopodia and degranulation [31]. In parallel, DV increases the expression of surface P-selectin and fibrinogen binding [31]. Dengue viral RNA and viral-like particles have also been detected in platelets of affected patients [14]. The same group also demonstrated a low level of DV production in platelets [36], suggesting that DV may be capable of replicating in anucleate platelets through mechanisms that are yet to be established. Whether or not DV is harbored and/or propagates in platelets, contributing to platelet dysfunction and

thrombocytopenia, deserves further consideration. New studies are also required to determine if active receptor-mediated DV entry occurs in platelets. These studies, and others, will go a long way in dissecting the functional role of dengue uptake into platelets and determine the contribution of platelets to organism defense or DV transport and dissemination of infection.

Soluble mediators

Key mediators that activate platelets and induce thrombocytopenia are often present in dengue infection. Monocytes from a donor infected with DV-1 respond to a second hit of DV-2 by generating Platelet Activating Factor (PAF) [37], a lipid mediator that augments platelet aggregation [38]. This observation is in agreement with others demonstrating that thrombocytopenia and disease severity is reduced in mice lacking the PAF receptor (PAFr) [39].

Fibrin degradation products (D-dimers) and thrombin/antithrombin complexes are typically increased after dengue infection [12,40]. von Willebrand factor is likewise increased [29,41], creating a milieu for enhanced platelet activation. An array of cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are also produced during dengue infection. These cytokines have been linked to the onset and regulation of thrombosis and hemostasis [40] and work from our group demonstrates that increased TNF-α and IL-1 β in dengue patients correlates with thrombocytopenia [6].

Targeting platelets in the treatment of dengue infection

Although thrombocytopenia is frequently observed in patients with dengue, severe bleeding is rare. When it occurs, however, excessive bleeding is associated with a high lethality. It is controversial as to whether the intensity of thrombocytopenia predicts bleeding risk in dengue patients; nonetheless, it is well accepted that severe thrombocytopenia associates with hemorrhagic manifestations. In addition, it is probable that other factors, such as disseminated intravascular coagulation (DIC), hepatic impairment and/or vascular dysfunction, act in concert with thrombocytopenia to induce bleeding.

Platelet transfusion has been used as a strategy for the prevention or treatment of severe bleeding in patients with dengue. However, recent WHO guidelines [2] do not recommend platelet transfusion for hemodynamically stable patients with thrombocytopenia. Even in patients who exhibit severe bleeding and hemodynamic instability, transfusion of platelets is only considered with restrictions. Importantly, these recommendations are based solely on the opinions of experts or small observational studies rather than randomized clinical trials.

Other treatments considered for dengue-induced thrombocytopenia include immunoglobulin (IVIG), intravenous anti-D immune globulin (anti-D) and PAFr antagonists [26,39,42]. Of these, anti-D has shown promise in the treatment of severe thrombocytopenia in DHF patients while a PAFr antagonist relieved thrombocytopenia in a mouse model of dengue infection [42]. By contrast, IVIG did not hasten the recovery of thrombocytopenia in dengue patients with secondary DV infection [26].

Moving forward, it will be important to consider nontraditional roles of platelets in the treatment of dengue. This includes their role in regulating viral infection and replication, inflammation and vascular integrity, which may identify new molecular targets for the treatment of dengue infection.

Conclusions

Dengue is the most common vector-borne viral disease in the world. It has also been recently reported in Florida [43,44] indicating that sustained transmission of dengue within the United States is a possibility. Although mitigating and controlling dengue outbreaks remains a high priority, an increased understanding of the disease is of paramount importance. One area of investigation involves understanding dengue pathogenesis in more detail, including the mechanisms by which dengue alters platelet behavior and, as a result, induces thrombocytopenia in the clinic. This review highlights our basic knowledge base regarding dengue and platelets, which is arguably scant in nature and deserving of more in-depth investigation. Commitment to this investigative front will undoubtedly identify new hemostatic and nonhemostatic roles of platelets in dengue infection.

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