Pathogenesis of Dengue Thrombocytopenia: Prospective Challenges for Researchers

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Introduction
The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people – over 40% of the world’s population – are now at risk from dengue. WHO currently estimates there may be 50-100 million dengue infections worldwide every year. Dengue Haemorrhagic Fever (DHF) affects most Asian and Latin American countries and has become a leading cause of hospitalization and death among children in these regions. There continues to be little emphasis on fundamental research on the genetic susceptibility of the host, pathogenesis of Dengue virus (DENV) associated thrombocytopenia, bleeding and plasma leakage [1].

DENV Associated Bleeding
Following DENV infection, bleeding can be fatal and severe thrombocytopenia contributes to it. Recently there have been reports of successful control of massive bleeding due to thrombocytopenia in two DENV patients with pulmonary and gastrointestinal bleeding [2]. Moreover, DENV infection can even lead to bone marrow failures [3] with a suppression and complete destruction of bone marrow cells. In addition, DHF can be associated with hemophagocytic lymphohistiocytosis, characterized by benign generalized histiocytic proliferation with marked hemophagocytosis. In such cases, levels of ferritin, and LDH are high along with, hypertriglyceridaemia and hypofibrinogenemia [4].

During 2010 in eight children who died with DENV infection the disseminated intravascular coagulation was the most common cause of death [5]. In one patient a primary DENV infection, who had been splenectomised for chronic autoimmune thrombocytopenic purpura, the haemorrhagic manifestations improved with high doses of corticosteroids [6]. On the contrary, in a woman with an identical picture in Guadeloupe, intravenous immunoglobulin was not useful [7].

DHF and the Genetic Makeup of the Host
The contribution through the genetic makeup of the host towards severity of DENV infection is noteworthy. In ethnic Thais with a secondary DENV infection, a variety of HLA class I alleles (HLA-A 0203, 0207, A 11, B 15, B 44, B 46, B 48, B 51, B 52), dendritic cell-specific intracellular molecule-3 Grabbing Non-intergrin (DC-SIGN), promoter polymorphisms and the AB blood group, were independently associated with either a susceptibility or a resistance to DENV infection and a more severe dengue haemorrhagic fever (DHF). Furthermore, the allelic variants of multiple gene loci involved in both acquired and innate immune responses contributed significantly to DENV disease outcome including the more severe dengue haemorrhagic fever [8].

Platelets and dengue virus
Fundamental studies would be needed to evaluate events during the interaction between DENV and platelets since an in vitro exposure of platelets from healthy donors to a cell culture-adapted dengue virus isolate was associated with platelet activation, an increased P-selectin expression and fibrinogen-binding property. The images obtained with atomic force microscopy, electron microscopy and flow-cytometry indicated an altered platelet membrane architecture, degranulation, and presence of filopodia and dilatation of the open canalicular system [9].

Host Factors and DENV Associated Haemorrhagic Manifestations
Several host factors appear to contribute towards DENV associated haemorrhagic manifestations though their precise contributions are still to be delineated.

Angiopoietins
The angiopoietin-1 (Ang-1) is stored in platelets and activates the endothelial cell-specific tyrosine kinase receptor Tie-2, which in turn leads to enhanced endothelial cell survival and stabilization and maintains vascular integrity. On the other hand, the angiopoietin-2 (Ang-2), is derived from endothelium and stored in Weibel-Palade bodies (WPBs), the storage granules of endothelial cells of blood vessels and heart. WPBs store and release von Willebrand factor and P-selectin, and promotes vascular leakage. They appear to be involved during transient plasma leakage in DHF/DSS.

In Indonesia, angiopoietin-1 and angiopoietin-2 levels and plasma leakage markers were monitored in a cohort of children with DHF/DSS along with. Patients with DHF/DSS had reduced angiopoietin-1 and increased angiopoietin-2 plasma levels on the day of admission when compared with levels at discharge and in healthy controls. There was an inverse correlation between angiopoietin-1 and markers of plasma leakage and a positive correlation between angiopoietin-2 and markers of plasma leakage [10].

Endothelial cell activation
In 73 Indonesian children with DHF and 30 with DSS, an endothelial cell activation was accompanied by an exocytosis of Weibel-Palade bodies (WPBs) and increasing circulating levels of WPB constituents, namely the von Willebrand factor antigen (VWF:Ag), VWF propeptide, and osteoprotegerin (OPG) while there was a decrease in, activity of the VWF-cleaving enzyme, ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin-1-like
domains). The VWF activation factor was also higher in children with DHF/DSS; it was highest in children who died. In all probability, high circulating levels of VWF in an active conformation, together with low ADAMTS-13 levels, contribute to the thrombocytopenia and complications of dengue [11].

**Plasminogen activator inhibitor**

In 71 children with DHF and 30 healthy children at Semarang in Indonesia, the level of Plasminogen-activator inhibitor 1 (PAI-1), which activates plasminogen resulting in fibrinolysis, transforming growth factor-β1 (TGF-β1), platelet count, liver function test and plasma leakage were monitored serially. The PAI-1 levels was higher in children with DHF on admission than on the second day of hospitalization. On the other hand, the levels of transforming growth factor-β1 (TGF-β1), that controls proliferation and differentiation of cells, were lower in cases than in controls on admission but on the second day increased towards the controls. PAI-1 values correlated well with platelet counts, plasma leakage, serum albumin on admission and the degree of pleural effusion. There was no correlation between mildly elevated liver function tests and PAI-1 levels [12].

**Point-of-care assays of abnormal host factors in DENV infections**

The existing laboratory tests to monitor abnormal host response [10-12] are complex, costly and require sophisticated laboratory infrastructure and trained personnel. Researchers should standardize 1-2 step in vitro assays which can be carried out in non-academic, non-research health centres. Based on results obtained with such tests, it would be possible to select those cases who were likely to suffer from DHF/DSS.

**Prospective Therapeutic Interventions**

The latest reports have shown that intravenous Anti-D immunoglobulin is effective in increasing platelet counts in patients with dengue and refractory thrombocytopenia [13]. Just now, a randomized, double-blind, placebo-controlled trial for adult patients with dengue has been approved with lovastatin since its anti-inflammatory effects on the endothelium have been advantageous. It would restore or improve endothelial cell function by an increased production of nitric oxide, a reduction of cytokines and acute phase proteins, resulting in reduction of inflammation in vessel wall [14]. Moreover, it is possible that lovastatin may have an anti-viral effect against dengue. Lovastatin has a good safety profile and with a low cost may well emerge as an attractive therapeutic candidate [15].

The recent progresses in virtual screening and docking techniques have led to the development of a new class of DENV inhibitors, the entry inhibitors since DENV entry into cells is through receptor-mediated endocytosis and the DG-SIGN present on dendritic cells is considered to be the most important viral receptor. This class of compounds has great potential to be used either alone or in combination therapy with viral replication inhibitors. It has been shown that entry inhibitors can prevent antibody-dependent enhancement ADE in human cells and subsequently immune activation [16].

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**References**


