

Theme Issue Article

Plasma leakage in dengue haemorrhagic fever

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Summary

Dengue viruses (DENV), a group of four serologically distinct but related flaviviruses, are the cause of one of the most important emerging viral diseases. DENV infections result in a wide spectrum of clinical disease including dengue haemorrhagic fever (DHF), a viral haemorrhagic disease characterised by bleeding and plasma leakage. The characteristic feature of DHF is the transient period of plasma leakage and a haemorrhagic tendency. DHF occurs mostly during a secondary DENV infection. Serotype cross-reactive antibodies and mediators from serotype cross-reactive Dengue-specific T cells have been impli-

cated in the pathogenesis. A complex interaction between virus, host immune response and endothelial cells likely impacts the barrier integrity and functions of endothelial cells leading to plasma leakage. Recently the role of angiogenic factors and the role of dengue virus on endothelial cell transcription and functions have been studied. Insights into the mechanisms that confer protection or cause disease are critical in the development of prophylactic and therapeutic modalities for this important disease.

Keywords

Dengue viruses, Dengue haemorrhagic fever, plasma leakage, permeability, immune system

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Introduction

Dengue remains an important threat to public health worldwide. There has been a significant increase in dengue cases reported to the WHO from approximately 900 cases per year from 1950 to 1959 to over 500,000 cases annually from 1990 to 1999 (1). It is estimated that over 50 million dengue virus (DENV) infections occur annually resulting in 500,000 hospitalisations and over 20,000 deaths. In addition to the known endemic countries in Southeast Asia where DENV infection causes significant mortality, morbidity and economic burden, dengue has become a threat in other areas of the world including the Americas, the Indian subcontinent and Oceania, making dengue one of the most important emerging infectious diseases worldwide (2–4).

Dengue viruses

Dengue is caused by infection with dengue viruses which are positive strand viruses belonging to the Flavivirus genus. Dengue viruses are transmitted through mosquito bites. *Aedes*

aegypti is the principal mosquito vector in virus transmission (5). The genome of DENV encodes 10 different gene products: C (capsid), prM (matrix), E (envelope), and non-structural proteins including NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (6). The E protein interacts with cellular receptor(s) which initiates viral entry. The amino acid sequences of the E proteins determine the antibody neutralising activity that classifies DENV into 4 serotypes: DENV1, 2, 3 and 4 (5). Non-structural proteins of DENV function in RNA replication and assembly and in viral protein processing. NS3 is a multifunctional protein with helicase and protease activities. Its serine protease activity requires NS2B as a cofactor (6). NS5 functions as an S-adenosine methyltransferase and RNA-dependent RNA polymerase. In addition to their roles in viral replication, some non-structural proteins also play a role in modifying host immune system. NS2A, NS2B and NS4B have been shown to interfere with type I IFN signalling pathway (7, 8). NS5 has been demonstrated to induce IL-8 production (9). NS1 is the only non-structural protein with a soluble form that can be detected in circulation (10).

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Clinical dengue

In endemic areas, infections occur early and the majority of children have been infected at least once in the first decade of life. Most primary (i.e. initial) infections in children are clinically inapparent although some may develop undifferentiated fever. Primary infections in older children and adults are more likely to cause dengue fever (DF), a febrile illness accompanied by a combination of non-specific symptoms including headache, retroorbital pain, myalgia and occasionally haemorrhagic manifestations (11). A minority of patients develop dengue haemorrhagic fever (DHF), the most severe form of dengue disease. The hallmark of DHF is the presence of plasma leakage which can lead to the loss of intravascular volume and circulatory insufficiency (11). Significant bleeding is another clinical feature associated with severe disease. Bleeding is common in both DF and DHF; more severe bleeding, particularly bleeding from the gastrointestinal tract, is found more frequently in DHF than in DF. Increased liver enzymes and thrombocytopenia are commonly observed in both DF and DHF cases but are more severe in DHF cases. Table 1 shows the World Health Organization case definitions of DF and DHF. The case definition of DHF is 1) fever, 2) bleeding, 3) thrombocytopenia (platelet count < 100,000 cells/mm³, 4) evidence of plasma leakage as indicated by the presence of pleural and/or ascitic fluid or haemoconcentration (11). DHF patients who have narrow pulse pressure (less than 20 mmHg) or show signs of shock are classified as dengue shock syndrome (DSS). Other severe clinical manifestations including hepatic failure and encephalopathy have been reported in dengue cases (12–14).

Current clinical classification of dengue illness describes DF and DHF as distinct clinical entities. DF and DHF share certain clinical features including fever, haemorrhagic tendency and, to a certain extent, thrombocytopenia. The clinical feature distinguishing DHF from DF is the presence of plasma leakage in DHF. Haemoconcentration, defined as a 20% increase in haematocrit, is widely used as an indicator of plasma leakage. However, haematocrit readings can be affected by factors other than plasma leakage such as fever, dehydration and haemorrhage. Furthermore, failures to obtain repeated measurements needed to calculate the degree of haemoconcentration often lead to dif-

ficulties in classifying dengue cases. Studies employing chest radiographs or serial ultrasonography to detect plasma leakage directly have demonstrated that progressive and significant accumulation of fluid only occurred in a subset of dengue cases (15, 16). The extent of plasma leakage has been shown to correlate with the decline in platelet counts (17). Despite the clinical classification of DF and DHF as distinct entities, they are likely a continuum of the same disease process with divergent outcomes with regard to the perturbation of vascular integrity.

Clinical course of DF and DHF

Figure 1 depicts the typical clinical course of patients in DF or DHF. Patients with DF or DHF usually present with a history of abrupt onset of high, persistent fever (18). Other clinical manifestations during the febrile phase of the illness include myalgia, nausea, vomiting, and abdominal pain. During this period, patients may display varied degrees of haemorrhagic tendencies ranging from small petechiae to nose bleed to bleeding from the gastrointestinal tract. Significant dehydration may also develop at this stage of illness, which may require intravenous fluid treatment. The febrile period can last between 2 and 7 days. Around the time of defervescence, DHF patients develop localised plasma leakage manifested as accumulation of fluid in pleural and abdominal cavities and haemoconcentration. The leakage lasts approximately 48 hours and is followed by a spontaneous and rapid resolution. The extent of plasma leakage varies between individual patients and can lead to intravascular volume depletion requiring fluid resuscitation. In addition, hepatic failure and encephalopathy may develop secondary to prolonged shock. Mortality is usually due to a delay in the recognition and treatment of plasma leakage.

Treatment of dengue

Close observation for signs of bleeding and circulatory compromise and prompt supportive treatment are the mainstay in dengue case management (18). Volume depletion often occurs due to fever, poor oral intake, bleeding, and plasma leakage. In cases with significant dehydration or depleted intravascular volume due to plasma leakage, intravenous fluid treatment with

Table 1: The World Health Organization (WHO) case definitions of dengue fever and dengue haemorrhagic fever.

Dengue fever (DF)	Dengue haemorrhagic fever (DHF)
Probable DF is an acute febrile illness with two or more of the following: <ul style="list-style-type: none"> – Headache – Myalgia – Arthralgia – Retro-orbital pain – Rash – Haemorrhagic manifestations – Leukopenia; and Supportive serology or occurrence at the same location and time as other confirmed cases of dengue. Confirmed DF is a case confirmed by laboratory criteria (serology, viral isolation, viral genome detection).	DHF case definition (all 4 components must be met) <ol style="list-style-type: none"> 1) Fever or history of fever, lasting 2–7 days, occasionally biphasic. 2) Haemorrhagic tendencies. 3) Thrombocytopenia (100,000 cells per mm³ or less) 4) Evidence of plasma leakage manifested by at least one of the following: <ul style="list-style-type: none"> – a rise in the haematocrit equal or greater than 20% above average for age, sex and population – a drop in the haematocrit following volume-replacement treatment equal to or greater than 20% of baseline – signs of plasma leakage such as pleural effusion, ascites, and hypoproteinaemia. Definition of dengue shock syndrome (DSS): DHF cases with documented narrow pulse pressure (< 20 mmHg), hypotension or other signs of shock.

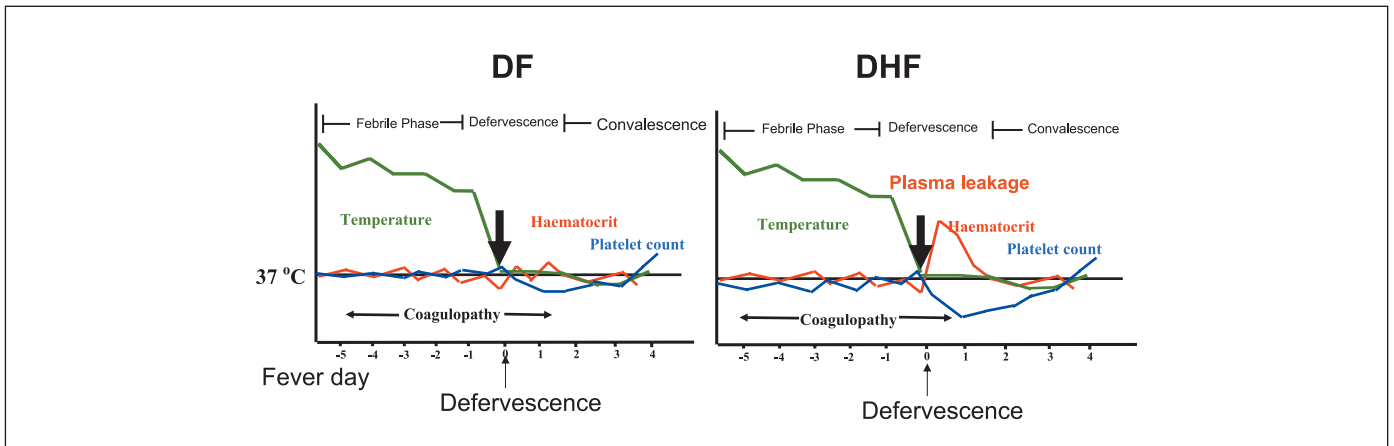


Figure 1: Clinical course of DF and DHF. An abrupt onset of high, persistent fever is an early manifestation of both DF and DHF. During the febrile phase, patients may develop other signs and symptoms including headache, myalgia and variable degrees of haemorrhagic tendency. Platelet counts decline during this stage, reaching the lowest point around the time of defervescence. During defervescence, some patients develop a transient increase in vascular permeability resulting in leakage

of albumin rich fluid in the pleural and abdominal cavities. This is associated with haemoconcentration as indicated by an increase in haematocrit. Evidence of plasma leakage and thrombocytopenia (platelet count $< 100,000/\text{mm}^3$) are the two criteria differentiating DHF from DF. The plasma leakage usually resolves after 48 hours followed by a convalescence period.

crystalloid solution is needed. Blood transfusion may be needed if haemorrhage is significant. In cases with shock, crystalloid fluid (10–20 ml/kg) is administered intravenously to maintain blood pressure. Colloid fluid has been used for resuscitation in shock cases with poor response to crystalloid fluid resuscitation. However, a study has demonstrated that colloid may not be superior to crystalloid fluid for this purpose (19). Intravenous fluid treatment must be carefully adjusted to adequately maintain circulation. Excessive fluid treatment can lead to serious complications such as pulmonary oedema and respiratory failure (18).

Risk factors for DHF

Prospective cohort studies in school children have demonstrated an increased risk of DHF in individuals with secondary infection compared to those with primary infection (20, 21). The risk of developing DHF in secondary versus primary infection varies between studies but is approximately 10-fold or more (4, 20). It is postulated that antibodies elicited by the first (or primary) exposure to one serotype of DENV, rather than protecting against infection by a second dengue virus of a distinct serotype, enhance virus uptake possibly via Fc receptors and promote viral replication. *In vitro*, virus incubated with diluted immune plasma can promote viral replication in monocytes and susceptible cell lines (22). Enhanced viral replication has been observed in rhesus monkeys that received immune serum prior to virus inoculation (23).

In addition to host immune status, various gene loci have been reported to be associated with the clinical severity of dengue disease. In connection with T cell immunity, certain HLA class I and class II loci have been linked to DHF while others have been associated with DF (24). Other genetic polymorphisms reported to be associated with disease severity in-

clude tumour necrosis factor (TNF)- α , Fc receptor, TAPs and DC-SIGN (CD209) (25–27).

Major outbreaks of DHF have followed the introduction of a new strain or new serotype of DENV into an area. This may be due to the immunologic susceptibility of the population to infection by newly introduced viruses. Intrinsic virulence of the viruses may also play a role in determining the disease severity. Outbreaks of DHF in the 1980-1990's in the Americas were associated with the introduction of Southeast Asian strains of D2V which replaced the D2V strains that previously existed in the region (28). Subsequent studies demonstrated that sera from individuals in the region who had been exposed to DENV-1 exhibited cross-neutralising activity against the American strain of DENV-2 but not the Asian strain (29, 30). In addition, Asian D2V variants replicated more effectively in primary human cells than American D2V strains *in vitro*. This has been attributed to differences in non-coding as well as coding portions of viral genomes (31, 32). Recent studies have demonstrated that viral NS proteins can interfere with type I IFN signalling and induce cytokine production (7–9). Whether these NS proteins contribute to the virulence of dengue virus and whether there are differences in genes encoding these proteins of various dengue viral isolates of different virulence awaits further studies.

Animal models

Understanding the pathogenesis of dengue has been hampered by the lack of animal models. Although non-human primates can be infected with DENV, they do not develop disease. Recent progress has been made in modeling dengue in mice. Studies using mouse-adapted strains of dengue virus and studies using mice genetically deficient in innate or adaptive immunity have demonstrated viral replication and some clinical features resem-

bling dengue disease including the presence of thrombocytopenia (33–35). Immunodeficient mice engrafted with human bone marrow cells and subcutaneously inoculated with dengue virus showed viral replication in various tissues and developed features of DHF including fever, thrombocytopenia and skin erythema (36, 37). Similar to human dengue, monocytes/macrophages appeared to be the main cells infected in these models. Infection of endothelial cells has also been reported. Signs of increased vascular permeability have been reported in intestine, liver and spleen in some models (35). The anatomical pattern of plasma leakage in these models does not resemble the pattern observed in DHF in humans (pleural effusion, ascites). Nevertheless, these models may be helpful in studying certain aspects of dengue pathogenesis such as the haematological changes and liver disease, and the role of innate and adaptive immunity in regulating viral replication.

Pathology and pathogenesis

Few studies exist that describe the pathology of DHF. In a study that described pathology findings in 100 fatal dengue cases, diffuse mucosal haemorrhage and serous membrane oedema were the two prominent gross findings (38). Microscopic findings included haemorrhage in various organs and perivascular and interstitial tissue oedema. Perivascular mononuclear infiltration and endothelial cell pyknosis were observed in some cases. Subsequent studies have demonstrated dengue antigen in various cell types including monocytes, Kupffer cells, alveolar macrophages, peripheral blood and splenic lymphocytes, and endothelial cells in the liver and the lungs (39).

Much of the present understanding of DHF pathogenesis is based on information obtained from studies in dengue patients and from *in vitro* models. *In vivo* monocytes/macrophages and lymphocytes are the main cells that are infected with DENV (39). Some studies have reported antigen staining in hepatocytes and endothelial cells (39–41). Although not formally demonstrated *in vivo*, dendritic cells are likely to be infected and play a key role in initiating immune response. A C-type lectin molecule expressed by dendritic cells (DC-SIGN, CD209) has been demonstrated by electron microscopy to bind to glycans of the E protein and play a role in viral uptake (42–44). Studies have shown that immature dendritic cells can be infected with DENV and the infection induced dendritic cell maturation (45, 46). Studies using skin explants have demonstrated that dendritic cells in the skin can be infected with locally inoculated DENV (47, 48).

The increased risk for DHF during secondary infections suggests that preexisting non-neutralising cross-reactive antibodies may enhance viral uptake by host cells leading to more viral replication. A number of studies have shown higher viral load in patients with DHF compared to patients with DF (49, 50). The levels of circulating NS1 protein were also found to be higher in patients with more severe disease (51). DENV for the most part does not cause death of endothelial cells. However, soluble NS1 protein has been shown to activate complement and may cause plasma leakage (10). The kinetics of viral load which peak at the time of presentation (febrile phase) and rapidly declines at the time of defervescence and plasma leakage suggests that a direct effect of virus on vascular permeability is not likely. Rather, the

increase in permeability likely occurs as a consequence of virus induced host responses. DENV-infected cells have been shown to produce a number of pro-inflammatory cytokines including TNF- α , IL-6, IL-8 and other chemokines. (9, 46, 52–54). Elevated levels of these mediators have been documented in DHF cases (55, 56).

Studies comparing the magnitude of T cell response during and after DENV infections have demonstrated more intense activation in patients with DHF compared to patients with DF both in terms of activation markers and the magnitude of T cell expansion (57–59). Studies have shown that CD8⁺ T cells specific to DENV serotype of a prior infection appear to be preferentially expanded during a secondary infection (59). Analysis of the functional phenotypes of CD8⁺ T cells in DHF cases have revealed that cross-recognition is associated with reduced cytolytic potential without much effect on cytokine production (60, 61). Further, activation with peptide variants has been shown to induce different sets of cytokines when compared to stimulation with the proband peptide in both CD4⁺ and CD8⁺ T cells (62–64). Cytokines and chemokines induced by suboptimal activation of T cells may have effects on vascular permeability leading to plasma leakage in DHF.

A series of studies have suggested that DENV-induced auto-antibodies play a role in the pathogenesis of DHF. A number of studies have reported that human and mouse antibodies to NS1 bind to host cells including endothelial cells and platelets (65–69). Antibody-binding to endothelial cells leads to apoptosis of these cells (66). In contrast, binding to platelets leads to activation of platelets (69). The target molecules of these antibodies have not been identified. Passive transfer of these antibodies into mice has induced various changes including bleeding and coagulopathy, elevated liver enzyme levels and endothelial cell death which appeared to be nitric oxide and caspase dependent (65–67). Since DHF is self-limited and patients usually recover rapidly without any sign of autoimmune diseases, the role of these antibodies in the pathogenesis of DHF in humans remains unclear.

Thus, both host and viral factors are involved in determining the severity of dengue illness. Pre-existing antibodies and host genetic susceptibility (such as the polymorphism in DC-SIGN or Fc receptor) influence viral uptake and replication which in turn induces an intense activation of both the innate and the adaptive immune systems. In particular, an aberrant or suboptimal activation of cross-reactive dengue-specific T cells may lead to poor viral clearance and the production of mediators with pro-inflammatory and vasoactive functions. Mediators released by T cells and by virus infected cells in combination with complement activation by viral proteins and immune complexes lead to an increase in vascular permeability (Fig. 2).

The role of endothelial cells in DHF

Although DENV can clearly infect human endothelial cell lines *in vitro*, conclusive evidence of DENV infection of endothelial cells *in vivo* is lacking. Dengue antigen but not genome was detected in endothelial cells in the liver sinusoids and the alveoli in human autopsy samples (39). A recent study has demonstrated the presence of NS3 protein in endothelial cells in the spleen but

not in other organs (40). Scarcities of human autopsy studies, the limited panels of tissues examined, and the time of specimen collection, which was usually after peak viraemia in most studies, have made it difficult to conclusively determine the infection of endothelial cells by DENV. In several recent studies in mice, dengue antigen has been detected in endothelial cells in infected mice (33, 70). In humans, swelling of endothelial cells but not extensive endothelial cell death or vasculitis has been reported (38). Apoptosis of endothelial cells in the lungs and intestinal mucosa in fatal DHF cases has been demonstrated in one human autopsy study but the extent of apoptosis was not quantified and appeared to be rather limited (41). Apoptosis of endothelial cells has been demonstrated in mice and has been proposed to be the mechanism of vascular leakage (65, 71).

Transcriptional activity, protein production and cell surface protein expression by endothelial cells are significantly altered by DENV. Several pathways which maybe involved in the pathogenesis of DHF are affected including inflammation, apoptosis and coagulation (72). Several chemokines are produced by DENV-infected endothelial cells *in vitro* such as MCP-1 (monocyte chemoattractant protein-1), RANTES and interleukin (IL)-8 (52, 56, 73). DENV also alters the production of coagulation factors by endothelial cells. Up-regulation of tissue plasminogen, thrombomodulin, PAR-1 (protease activated receptor-1) receptor and tissue factor receptor, and down-regulation of tissue factor inhibitor and activated protein C have been reported. (74–77). Consistent with these findings, elevated levels of tissue factor and thrombomodulin have been found in DHF cases (78).

Expression of cell surface molecules of endothelial cells is affected by DENV. Expression of ICAM-1 (intercellular adhesion molecule-1) and beta-3-integrin on microvascular endothelial cell lines has been reported (79–81). Interestingly, blocking of beta-3 integrin expression or blocking its functions by antibodies inhibits DENV entry and replication in microvascular endothelial cells, suggesting that DENV infection may en-

hance subsequent rounds of virus entry into endothelial cells by over-expressing beta-3 integrin. Expression of cytokine receptors is also affected by DENV. Infection of human umbilical vein endothelial cells (HUVEC) results in a decrease in the soluble form of VEGF (vascular endothelial growth factor) receptor2 (sVEGFR-2) in culture supernatants and a concomitant increase in membrane expression of VEGFR-2 (82). This effect is virus dose dependent. Plasma levels of sVEGFR-2 in DHF patients progressively decline during the course of the illness and inversely correlate with the plasma viral load, providing an *in vivo* correlate for the *in vitro* findings. This decline was associated with a simultaneous increase in plasma free VEGF. These findings suggest that DENV-induced changes in the expression of receptors for permeability enhancing mediators may be a mechanism involved in plasma leakage in DHF.

Interactions between the immune system and endothelial cells in DHF

Monocytes, macrophages and dendritic cells are the major targets for DENV. Infection of these cells results in the production of mediators that may affect the functions of endothelial cells. Supernatant from DENV-infected monocytes induces permeability changes in the HUVEC monolayer. This is due partly to TNF- α (53). A study has demonstrated that infection with DENV up-regulates the expression of matrix metalloprotease enzymes, MMP-2 and MMP-9, by dendritic cells (83). HUVEC monolayers treated with culture supernatants from infected dendritic cells exhibit enhanced permeability in association with a down regulation of surface platelet endothelial cell adhesion molecule-1 (PECAM-1) and VE (vascular endothelial)-cadherin expression and F-actin re-organisation. Injection of culture supernatants from DENV-infected dendritic cells also caused localised plasma leakage and bleeding *in vivo*. DENV infection of endothelial cells has been reported to up-regulate MMP-2 and

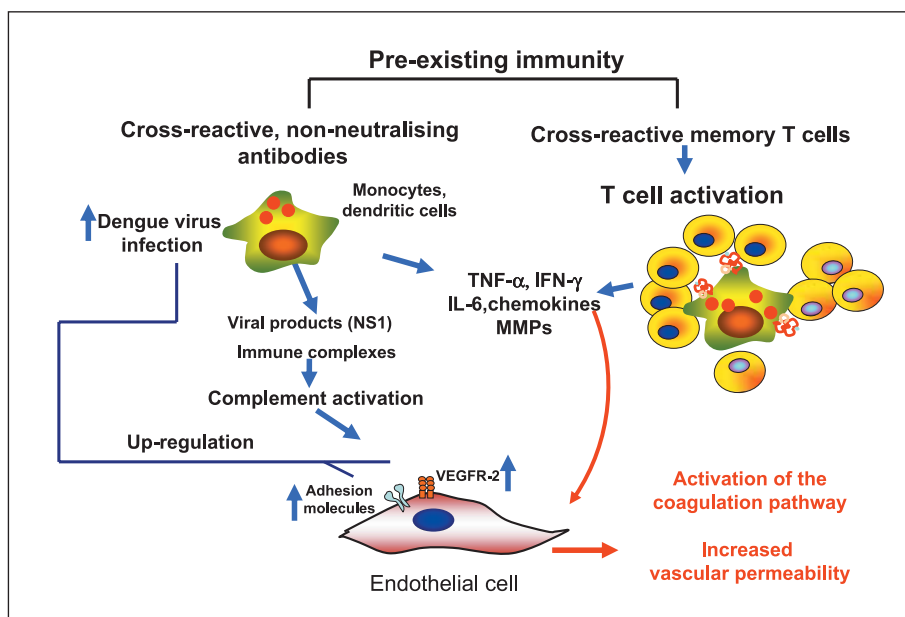


Figure 2: Immunopathogenesis of DHF.

Primary exposure to dengue virus induces both humoral (antibodies) and cellular (T cells) mediated immune responses. During a secondary infection with a different dengue virus serotype, cross-reactive, non-neutralising antibodies bind to virus and enhance viral uptake via Fc receptors resulting in enhanced viral replication and higher antigen load which lead to an exaggerated activation of cross-reactive dengue-specific T cells. Dengue virus may have direct effects on endothelial cells such as modulation of cell surface molecule and cytokine receptor expression. Biological mediators released by T cells and by virus-infected cells along with complement activation by viral proteins and immune complexes, may result in enhanced vascular permeability and coagulopathy.

increase permeability (84). It has been postulated that increased MMPs by DENV-infected cells may be a mechanism of vascular leakage in DHF. However, the general lack of structural changes of vessels in histology studies of human cases seems to argue against this hypothesis.

The interaction between endothelial cells, DENV and immune cells has been examined in a study in which HUVECs were infected with DENV and co-cultured with naïve peripheral blood mononuclear cells (PBMCs) (85). Neither DENV infection nor PBMCs alone had an effect on the permeability of the HUVEC monolayer. However, increased permeability was observed when HUVECs were infected with live DENV and PBMCs were added. The effect of PBMCs was mediated by adherent, CD14⁺ cells indicating that macrophages are the critical cells. The increase in permeability was associated with a down-regulation of vascular endothelial cadherin expression.

In summary, a complex interaction between DENV, immune cells and endothelial cells impacts endothelial cell barrier function. DENV may affect endothelial cells directly or indirectly through mediators released from infected or activated immune cells. Changes in the expression of adhesion molecules, enzymes, and cytokine receptors on endothelial cells may lead to enhanced vascular permeability and the activation of the coagulation system in DHF (Fig. 2).

Remaining questions and future research

Many questions remain unresolved regarding the mechanisms of plasma leakage in DHF. DHF shares many of the clinical manifestations and pathogenesis with other viral haemorrhagic fevers such as fever, thrombocytopenia, haemorrhagic tendency and plasma leakage (86–88). Infection of the endothelium has been better documented and plays a more prominent role in the pathogenesis of other haemorrhagic fevers such as Hantaviruses and, to a lesser extent, Ebolavirus infection. Tissue destruction in Ebolavirus infection is attributed to direct viral effects since there is little tissue inflammation (87). Both tissue inflammation and tissue destruction in DHF are rather limited. Although several mediators with pro-inflammatory effects are reported to be elevated in DHF, these levels are elevated only relative to levels found in DF or normal controls. Therefore, the true contribution of these molecules in the pathogenesis of DHF is not known. The overall lack of tissue inflammation, the transient nature of plasma leakage and the rapid recovery in DHF patients suggest that mediators that regulate permeability with relatively little pro-in-

flammatory effects may play an important role in plasma leakage in DHF. These cytokines include those involved in vessel development such as VEGF and angiopoietins. Alternatively, the lack of inflammation in DHF may be due to mediators with anti-inflammatory or anti-chemotactic effects. Elevated levels of transforming growth factor-beta have been reported in DHF patients (89, 90). Whether DENV elicits other biological mediators with anti-inflammatory properties remains to be determined.

The predisposition for DHF during secondary infections implicates the adaptive immune system in the pathogenesis of DHF. This is in contrast to Ebolavirus infection in which innate immunity appears to play a major role (87). Adaptive immunity, particularly CD8⁺ T cells, has been demonstrated to be important in the pathogenesis of Hantavirus (91, 92). The adaptive immune arms that are involved in plasma leakage in DHF are not defined. However, the association between certain HLA alleles and disease severity suggests that T cells may be important in this process. Given the importance of DENV as a global and emerging public health problem, there are currently considerable efforts in developing vaccines against DENV. Therefore, it is imperative that the immune effectors that confer either protection or pathology be identified. Dissecting the effector functions related to protection or pathology will require animal models that can reproduce the clinical features of DHF. The precise delineation of the roles of various mediators in plasma leakage can only be done with genetically modified animals or by functional ablation of such mediators in animal models. Despite the recent progress in the development of animal models, studies in humans remain indispensable in the effort to gain further insights into this condition. Well designed, prospective studies of well characterised dengue cases are still instrumental in our understanding of this disease. Insights in vascular biology and the interplay between virus, the immune system and endothelium in DHF will be crucial for the development of predictive markers and therapeutic interventions for this condition.

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