


Pathogenesis of vascular leak in dengue virus infection

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Summary

Endothelial dysfunction leading to vascular leak is the hallmark of severe dengue. Vascular leak typically becomes clinically evident 3–6 days after the onset of illness, which is known as the critical phase. This critical phase follows the period of peak viraemia, and lasts for 24–48 hr and usually shows rapid and complete reversal, suggesting that it is likely to occur as a result of inflammatory mediators, rather than infection of the endothelium. Cytokines such as tumour necrosis factor- α , which are known to be elevated in the critical phase of dengue, are likely to be contributing factors. Dengue NS1, a soluble viral protein, has also been shown to disrupt the endothelial glycocalyx and thus contribute to vascular leak, although there appears to be a discordance between the timing of NS1 antigenaemia and occurrence of vascular leak. In addition, many inflammatory lipid mediators are elevated in acute dengue viral infection such as platelet activating factor (PAF) and leukotrienes. Furthermore, many other inflammatory mediators such as vascular endothelial growth factor and angiopoietin-2 have been shown to be elevated in patients with dengue haemorrhagic fever, exerting their action in part by inducing the activity of phospholipases, which have diverse inflammatory effects including generation of PAF. Platelets have also been shown to significantly contribute to endothelial dysfunction by production of interleukin-1 β through activation of the NLRP3 inflammasome and also by inducing production of inflammatory cytokines by monocytes. Drugs that block down-stream immunological mediator pathways such as PAF may also be beneficial in the treatment of severe disease.

Keywords: dengue; lipid mediators; NS1 antigen; platelet activating factor; vascular leak.

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Introduction

Dengue viral infections represent one of the most rapidly emerging mosquito-borne infections in the world, spreading to many geographical regions and causing almost 100 million apparent dengue infections each year.¹ From 2005 to 2015, although the mortality rates due to many infectious diseases decreased, the deaths due to dengue increased by 48.7%, resulting in an estimated 18 400 deaths in 2015.² Although there is now a dengue vaccine, which is licensed to be used in individuals over 9 years of age in several countries, it is only recommended in countries with high rates of dengue seroprevalence, due to its varied efficacy.³ Intense monitoring with meticulous fluid control is currently the only option in the management of acute dengue viral infection, as specific treatments for dengue are not yet available.

Although infection with the dengue virus (DENV) is associated with a self-limiting illness in the majority of individuals, it can cause severe clinical disease manifestations such as dengue haemorrhagic fever (DHF) and organ involvement in a significant proportion of individuals.^{4,5} Patients with dengue may progress through three clinical phases known as the febrile phase, the critical phase and the recovery phase.⁶ Once infected with DENV, after an initial incubation period of typically 3–7 days, the infection manifests with a sudden onset of high fever, accompanied by high viraemia, which is known as the febrile phase.⁶ Some individuals proceed to the critical phase, which lasts for 24–48 hours and is associated with plasma leakage; whereas others directly proceed to the recovery phase without developing plasma leakage.⁷ Severe dengue is associated with a transient increase in vascular permeability due to endothelial dysfunction in the

critical phase.⁸ Increase in vascular permeability is associated with vascular leakage, resulting in accumulation of fluid in pleural and peritoneal cavities, and with reduction in blood pressure and pulse pressure, resulting in poor organ perfusion.^{5,8} The post-capillary venules have been shown to be the predominant site, where increased permeability due to vascular leak is observed.⁹ Changes in the microvascular circulation leading to reduced blood flow and perfusion have been shown to occur in patients who develop plasma leakage during the critical phase.¹⁰ Many cytokine mediators,¹¹ mast cell products,^{12,13} inflammatory lipid mediators^{14–16} and the disruption of the function of the endothelial glycocalyx by dengue NS1^{17,18} have been implicated in vascular leak. These will be discussed in turn.

Cytokines as a cause of vascular leak

Many pro-inflammatory and immunosuppressive cytokines and chemokines are elevated in patients with dengue viral infection.^{19–22} These pro-inflammatory cytokines were thought to be produced by highly cross-reactive T cells, which expand in secondary dengue infection, but are less effective in clearing the infecting virus serotype.^{23–25} However, subsequent studies have shown that DENV-specific T cells are present in low frequency or undetectable during the leakage phase of acute dengue,^{26,27} and produce minimal amounts of these cytokines.¹⁹ More recent studies suggest that DENV-specific T cells could in fact be protective.^{28,29} Instead it is more likely that other haematopoietic cells that are directly infected with the DENV such as dendritic cells, monocytes and macrophages are the predominant source of these cytokines through activation of innate immune pathways.^{30,31} Although many types of pro-inflammatory and immunosuppressive cytokines are seen at higher levels in patients with DHF when compared to those with dengue fever (DF),^{20,32} the levels of these cytokines are different during the febrile and critical phases.^{4,11,30} While immunosuppressive cytokines such as interleukin-10 (IL-10) decrease during the critical phase, cytokines that have been associated with vascular leak such as tumour necrosis factor- α (TNF- α), tend to increase.^{4,30} Although TNF- α and many other cytokines are also increased in severe forms of influenza virus infection, the levels of some of these cytokines are typically lower than those detected in patients with DHF.^{33,34} For instance, the median levels of TNF- α in patients with severe influenza infection were below 5 pg/ml,^{33,34} whereas the median levels of those with DHF have been reported to be well over 10 pg/ml during the critical phase, with levels > 40 pg/ml in some patients.^{19,30,32} Therefore, although vascular leak is not seen in infections such as influenza, which can be associated with a cytokine storm, the levels of many cytokines such as TNF- α can be much higher in dengue infection, which may contribute to vascular leak.

In vitro experiments have shown that sera from patients with DHF do disrupt the endothelial monolayer, resulting in morphological changes.¹¹ In dengue mouse models, use of TNF- α blocking antibodies was associated with improved survival and reduction in liver involvement,³⁵ suggesting that cytokines such as TNF- α could indeed be contributing to the vascular leak in acute dengue (Fig. 1). Interleukin-2 used in high doses has been shown to cause vascular leak syndrome in patients with renal carcinoma and melanoma.³⁶ However, the contribution of IL-2 in causing vascular leak in dengue is debatable, as some studies have shown that the IL-2 is elevated,³⁷ whereas others have shown that IL-2 is actually reduced.^{19,20}

Mast cell products associated with vascular leak

The importance of mast cells and their products such as cytokines and vascular endothelial growth factor (VEGF) in the pathogenesis of dengue is emerging.^{12,38,39} Levels of VEGF, chymase and tryptase were found to be higher in patients with dengue, especially in those with plasma leakage.^{12,38} One early study showed that 24-hr urine histamine content was elevated in patients with DHF⁴⁰ and also that in murine models the increased permeability of the blood–brain barrier was significantly reduced by anti-histamines in a dose-dependent manner.⁴¹ *In vitro* studies have shown that mast cells are directly infected with the DENV and also that antibody-dependent enhancement leads to increased mast cell degranulation in both *in vitro* and *in vivo* dengue mouse models.^{38,42} Although mast cells are infected with the virus, the virus titres needed to infect mast cells have been shown to be several fold higher than other DENV permissive cells such as monocytes and dendritic cells.⁴³ Therefore, DENV-specific antibodies are thought to play a significant role in secondary dengue infections, by forming immune complexes with the DENV and thereby enhancing infection of mast cells via Fc γ receptors.³⁸ This higher infection of mast cells is thought to lead to increased degranulation during secondary dengue viral infections, thereby leading to enhanced endothelial activation and vascular leakage.⁴³

Although we and others have found that levels of mast cell tryptase, which is a protease specific to mast cells, are elevated in patients with DHF when compared with those with DF, their levels are still usually within the normal range.^{12,16} Interestingly, the levels of tryptase and the activity of secretory phospholipase A2 (sPLA2) do associate with the degree of viraemia. However, the levels and the changes of serum mast cell tryptase in patients with primary or secondary dengue infection are similar. Others have found that although mast cell products such as VEGF are higher in patients with more severe forms of dengue, there is no difference in the levels of VEGF in those with primary and secondary dengue.⁴⁴ As dengue viral infection is characterized by rapidly changing clinical and laboratory

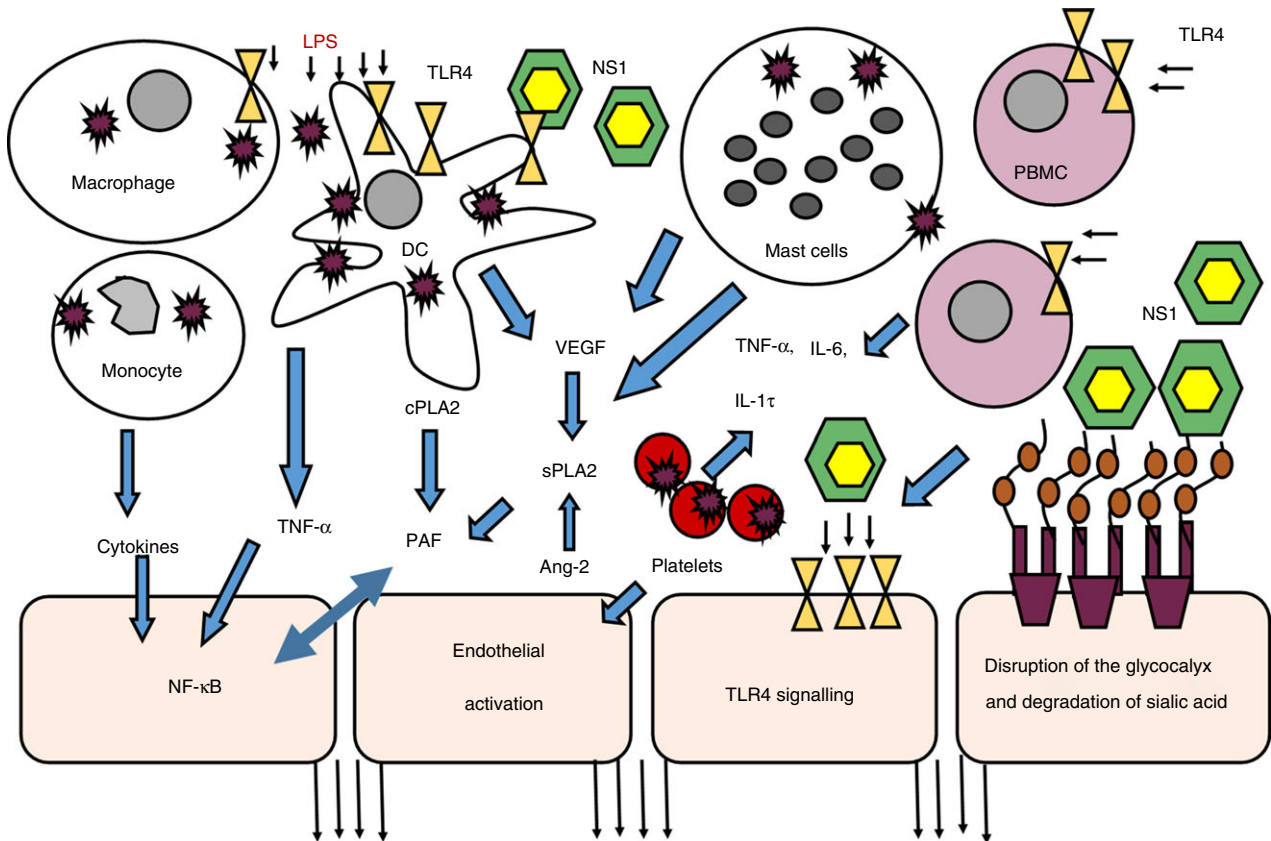


Figure 1. Model of endothelial dysfunction in dengue infection. Cytokines and tumour necrosis factor- α (TNF- α) produced by dengue virus (DENV)-infected monocytes, dendritic cells (DCs) and macrophages possibly cause endothelial activation and also activate nuclear factor- κ B (NF- κ B) to induce platelet-activating factor (PAF) production. LPS present in the blood by possible microbial translocation amplifies this effect and also induces PAF production from these cells. Vascular endothelial growth factor (VEGF) produced by mast cells and other cells act on secretory phospholipase A2 (sPLA2) to induce production of PAF and mast cells are also a possible source of sPLA2, which leads to PAF production. Platelets, which are also directly infected by the DENV, produce many cytokines by forming platelet monocyte aggregates. Platelet-derived microparticles, are an important source of interleukin-1 β (IL-1 β), which are produced by activation of the NLRP3 inflammasome. NS1 could also act through Toll-like receptor 4 (TLR4) inducing phospholipases to generate PAF from phospholipids and also induce production of many inflammatory cytokines, which contribute to the 'cytokine storm'. Ang-2 produced by endothelial activation also in turn activated sPLA2 to induce PAF production. Dengue NS1 hexamers bind to the endothelial glycocalyx, resulting in loss of sialic acid. NS1 also leads to the cleavage of heparan sulphate from the endothelial glycocalyx.

parameters, it would be important to evaluate several mast cell proteases and other mast cell mediators longitudinally during the course of illness in patients with DF and DHF and also in those with primary and secondary dengue viral infection. As VEGF is also produced by macrophages, endothelial cells, T cells and a variety of other cell types,⁴⁵ the role of mast cells as a source of VEGF in dengue, and their contribution to disease pathogenesis should be further evaluated. However, as early studies showed that histamine released from mast cells could be playing a role in vascular leakage,^{40,41} it would be important to also further investigate this mediator.

Lipid mediators

Platelet-activating factor (PAF) is a phospholipid that has many physiological functions and exerts its actions in

part by binding to a specific receptor known as PAF receptor.⁴⁶ It is a potent inducer of increased vascular permeability in sepsis and anaphylaxis⁴⁷ and has been shown to activate nuclear factor- κ B (NF- κ B), resulting in the expression of many inflammatory cytokines such as TNF- α and IL-1 β .^{48–50} PAF is rapidly released by many cells such as endothelial cells, leucocytes, mast cells, macrophages and monocytes by the action of both secretory phospholipases and cytosolic phospholipases on phospholipids.^{51,52} The PAF receptor is a G-protein-linked receptor and its expression is regulated by inflammatory responses and also by PAF itself.⁴⁶ PAF has been shown to be associated with vascular leak in murine models of dengue viral infection, which can be reversed by PAF inhibitors.⁵³ We recently showed that PAF was significantly elevated in patients with DHF, especially during the critical phase.¹⁴ Reduction in the expression of

the tight junction protein ZO-1 and reduction in trans-endothelial electrical resistance induced by sera from patients with acute dengue was significantly blocked by the use of PAF receptor blockade,¹⁴ suggesting that PAF is an important mediator of increased endothelial permeability in acute dengue.

Phospholipase A2s comprise a group of enzymes generating inflammatory free fatty acids including PAF precursors and also lysophospholipids; and play a major role in the systemic inflammatory response.^{54,55} The two major groups of phospholipase A2, which are the cytoplasmic phospholipase A2 and secretory sPLA2 are known to generate and regulate PAF.^{52,56} We found that the sPLA2 activity was markedly higher in patients who developed vascular leak when compared with those with DF, very early during the course of illness.¹⁶ VEGF, which has been shown to be elevated in those with DHF, is also known to induce the production of sPLA2s⁵⁶ (Fig. 1). It has been shown that the increase in vascular permeability due to VEGF was completely abolished by PAF receptor blockers *in vivo* in rodent models.⁵⁷ Therefore, VEGF could be contributing to increasing endothelial permeability through PAF.

Among the many subtypes of sPLA2s, group IIA predominates in serum and its activity has been shown to be induced by many inflammatory stimuli and bacterial products such as lipopolysaccharide (LPS).^{52,58} Microbial translocation has been shown to occur in dengue and higher LPS levels have been seen in those with vascular leakage when compared with those with DF.^{59,60} We found that LPS acts synergistically with the DENV to induce production of PAF and many other inflammatory cytokines such as TNF- α and IL-6,³⁰ thereby suggesting that LPS could contribute to the increased vascular permeability in acute dengue by inducing PAF, possibly through increasing activity of cPLA2 and sPLA2 (Fig. 1). As dengue NS1 has also been shown to act through Toll-like receptor 4 (TLR4) and induce cytokine production,¹⁸ it is possible that NS1 could contribute to the increase in sPLA2 activity through TLR4.

Interestingly, there is a striking pattern in PAF levels in patients with dengue, with PAF levels being high in the morning and almost undetectable in early afternoon. This pattern was more prominently observed in those with DHF.^{14,16} As changes in PAF levels have not been evaluated twice a day in other disease conditions such as sepsis and anaphylaxis, where high PAF levels are seen, it is not clear if this phenomenon is observed only in dengue viral infection. However, it has been shown that pro-inflammatory cytokines such as TNF- α , IL-1 β and interferon- γ , induce PAF production by monocytes in a bi-phasic manner.⁴⁹ PAF is known to activate NF- κ B, which regulates the production of many inflammatory cytokines. Therefore, activation of NF- κ B by PAF could in turn lead to production of TNF- α and IL-1 β , further leading to

more PAF production.⁶¹ Indeed, we have observed that the kinetics of changes in TNF- α and IL-1 β follow the same patterns as PAF in acute dengue.³⁰

Sphingosine -1-phosphate (S1P) is a signalling sphingolipid that enhances endothelial barrier integrity^{62,63} and also counteracts the effects of VEGF on the vascular endothelium.⁶² It has been shown to oppose the actions of PAF in murine models by inhibiting the extent of vascular leak and impairing the inflammatory response driven by PAF.^{64,65} We and others found that blood S1P levels were significantly lower in those with DHF, especially during the critical phase.^{15,66} Platelets are an important source of S1P⁶³ and we found that serum S1P levels significantly correlated with platelet counts in acute dengue.¹⁵ Therefore, reduction in platelet counts could contribute to a decrease in S1P levels, especially in the critical phase of dengue, leading to increase in the endothelial permeability.

Leukotrienes are generated by the action of lipoxygenase enzymes on arachidonic acid substrates, and include LTB₄, LTC₄, LTD₄ and LTE₄.⁶⁷ Apart from being pro-inflammatory mediators and potent chemoattractants, some are also known to increase vascular permeability and induce vascular leak.⁶⁷ Increased activity of LTB₄ has been observed in patients with acute dengue viral infection, and exposure of neutrophils to the DENV induced the production of LTB₄.⁶⁸ In addition, in experimental dengue mouse models, treatment with leukotriene receptor antagonists resulted in a significant reduction in plasma leakage in dengue-infected mice,³⁹ suggesting that leukotrienes may be playing an important role in fluid leakage in dengue.

Increase in endothelial permeability induced by dengue NS1

The NS1 protein of the DENV is a secretory lipoprotein that plays an essential role in viral replication intracellularly⁶⁹ and causes many effects on the immune system in secretory forms.^{17,18,70,71} It is present intracellularly as a monomer, on the cell membrane as a dimer and as a hexamer with a lipid rich core in the secretory form.⁷² Higher NS1 levels have been shown to associate with DHF⁷³ and the NS1 antigen has been shown to persist for a longer duration in those with vascular leak.^{70,74} DENV NS1 is known to interact with the complement system,⁷⁵ and activates many innate immune cells to produce inflammatory cytokines by acting through TLR4,¹⁸ and also induces immunosuppressive cytokines such as IL-10 from monocytes, thereby potentially contributing to disease pathogenesis.⁷⁰ NS1 was shown to induce a dose-dependent increase in the production of a number of pro-inflammatory cytokines such as IL-6, IL-1 β , TNF- α from peripheral blood mononuclear cells, acting through TLR4, which is likely to contribute to the 'cytokine

storm' associated with dengue.¹⁸ However, *in vitro* experiments carried out endothelial monolayers and in dengue mouse models suggest that NS1 could also play a role on the vascular endothelium and so induce vascular leak (Fig. 1).

In dengue mouse models, DENV NS1 of all serotypes increased the endothelial permeability in a dose-dependent manner, which was abrogated by anti-NS1 antibodies.¹⁷ Subsequent experiments showed that NS1 also disrupted the endothelial glycocalyx layer in human pulmonary vascular endothelial cells in a dose-dependent manner and that exposure to NS1 resulted in loss of sialic acid from the cell surface.⁷⁶ In addition, NS1 also induced shedding of the heparan sulphate proteoglycans, which contributed to disruption of the endothelial glycocalyx layer.⁷⁶ Binding of NS1 to endothelial monolayers *in vitro* was maximal between 3 and 12 hr and returned to normal 48 hr after treatment with NS1.⁷⁶

Studies carried out on patients with dengue have shown varied results regarding the presence of NS1 antigenaemia, its persistence and relation to clinical disease severity. Although we have found that patients who are likely to have NS1 antigenaemia beyond day 5 of illness, are likely to have more severe forms of dengue,⁷⁴ some studies have found that NS1 antigen levels, especially during days 4–8 of illness, were lower in patients with more severe forms of illness.⁷⁷ NS1 persistence and NS1 antigen levels found in acute infection have been shown to depend on the infecting DENV serotype, as those who are infected with DENV-1 were found to have higher NS1 antigen levels and NS1 persistence beyond day 5 of illness.^{77,78} A large study in Vietnam has shown that NS1 antigen levels were similar in primary and secondary infection and also in those with DF and DHF.⁷⁸ As NS1 antigen levels appear to be similar in those who develop vascular leak and in those who do not, the contribution of NS1 to vascular leak should be further evaluated. Similar to observations in humans, where vascular leak becomes clinically detectable around day 4–7 of illness,⁷ mice injected with NS1 developed vascular leak approximately 3 days later.¹⁷ Therefore, vascular leak often appears to occur at a point in the illness when the NS1 antigen levels are often lower. Therefore, although NS1 induced endothelial permeability in endothelial cell monolayers, which was maximal during the first 24 hr, the relevance of this *in vivo* in patients with dengue should be further evaluated due to the discordance of the timing of vascular leak and NS1 antigenaemia.

Endothelial infection and production of inflammatory mediators

In vitro studies have shown that endothelial cells can be directly infected by the DENV and produce many inflammatory mediators that lead to endothelial dysfunction.^{79–81}

In dengue mouse models, marked apoptosis of endothelial cells has been observed, which is thought to be due to direct infection of the cells and also due to TNF- α .^{82,83} It has also been shown using *in vitro* models that sera of patients with acute dengue resulted in endothelial cell apoptosis and lysis, which have been attributed to anti-NS1 specific antibodies causing direct damage through nitric oxide and complement-mediated damage.^{84,85} Vectors that expressed DENV proteases were also shown to induce endothelial cell apoptosis in dengue mouse models, by inducing NF- κ B.⁸⁶ However, autopsy studies have failed to show that endothelial cells are infected by the virus during dengue viral infection and have not demonstrated any endothelial damage.^{87,88} Moreover, antigen–antibody complexes or complement deposition were not seen in any of these cases.⁸⁷ The increase in endothelial permeability is transient in dengue, with the clinically evident fluid leakage phase lasting for 24–48 hr and the vascular leak completely ceasing following this phase. Therefore, as shown in autopsy studies in patients with dengue infection, it is questionable if significant direct endothelial infection by the DENV actually occurs *in vivo*.

Excessive endothelial activation⁷ has also shown to be an important cause of vascular leak through activation of NF- κ B.⁹ Excessive endothelial activation leads to the production of many inflammatory cytokines that lead to increased vascular permeability and inhibition of NF- κ B in sepsis mouse models, reduced vascular leak, reduced multi-organ failure and lower survival.⁹ Therefore, rather than direct infection of the endothelium leading to endothelial apoptosis and dysfunction, it is more likely that the DENV could be causing widespread activation of the endothelium through NF- κ B, which contributes to vascular leak.

Angiopoietin and vascular leak

Angiopoietin 1 (ang-1) and 2 (ang-2) act through their tyrosine kinase receptor to maintain endothelial integrity. Although ang-1 maintains endothelial barrier integrity, ang-2 has an opposing effect and increases endothelial permeability.⁸⁹ Ang-2, which is exclusively produced by the endothelium, was found to be higher in those with severe plasma leakage, whereas ang-1 levels were lower.^{89,90} However, a more recent study that evaluated the disturbances in the microcirculation at several time-points in clinical disease along with vascular endothelial activation markers found that although ang-2 was elevated in those with plasma leakage, the ang-2/ang-1 ratios and plasma vascular cell adhesion molecule 1 levels were similar in those who developed plasma leakage, when compared with those who did not.¹⁰

Both ang-1 and ang-2 are important regulators of PAF production by the endothelium. The two angiopoietins

were shown to induce PAF very rapidly in bovine endothelial cell lines in a bi-phasic manner.⁹¹ The induction of PAF production by endothelial cell lines was abolished by the use of sPLA2 inhibitors, implying that ang-1 and ang-2 stimulated PAF production through activation of sPLA2.⁹¹ As we found that sPLA2 activity was significantly higher in those who developed plasma leakage¹⁶ and as sPLA2s type V is known to induce PAF production by endothelial cells,⁹¹ endothelial activation leading to increased production of ang-2 could also be acting through sPLA2 to induce PAF (Fig. 1).

Platelets and vascular leak

Platelets in patients with dengue viral infection have been shown to express P-selectin, which is an adhesion molecule that facilitates platelet binding to leucocytes.⁹² Platelets are known to form platelet–monocyte aggregates that correlate with the presence of thrombocytopenia, and these complexes significantly associate with vascular leak.⁹² Such platelet–monocyte interactions induce cytokine production by monocytes^{93,94} and such platelet–monocyte interactions in dengue induce production of IL-1 β , IL-8 and IL-10 by monocytes.⁹² We and others have found that IL-10, IL-1 β , monocyte chemoattractant protein 1 and IL-8 levels were associated with severe dengue^{22,95} and that monocytes were likely to be the predominant source of IL-10.⁹⁶ Apart from interaction with monocytes, platelets are also known to contribute to vascular permeability due to production of IL-1 β by platelet microparticles.⁹⁷

High IL-1 β has been demonstrated in patients with severe dengue and is thought to associate with increase in vascular permeability.^{30,97,98} Platelet-derived microparticles were shown to be an important source of IL-1 β during dengue infection, which is thought to be generated by activation of the NLRP3 inflammasome.⁹⁷ Microparticles derived from platelets were shown to be enriched with the NLRP3 inflammasome in patients with dengue and reactive oxygen species have been shown to be responsible for the inflammasome activation.⁹⁷ As the proportion of IL-1 β -enriched platelets and microparticles was significantly higher in patients who developed vascular leak, IL-1 β could also be contributing to increased vascular permeability. Numerous studies have shown that platelets and megakaryocytes can be infected *in vitro* and also that active infection of platelets has been shown to occur in patients with acute dengue.^{99–102} Such infection of platelets may lead to thrombocytopenia and cytokine production, and so potentially contribute to severe clinical disease.¹⁰³

Apart from IL-1 β , it has been shown that serotonin released from microparticles of activated platelets also leads to vascular leak.¹⁰⁴ Although red-cell-derived microparticles were elevated in patients with DHF and

correlated with disease severity, platelet-derived microparticles were found to be significantly reduced.¹⁰⁵ Serum serotonin levels were found to be significantly lower in patients with acute dengue, especially in those with DHF.¹⁰⁶ Therefore, it is unlikely that serotonin is playing a significant role in the vascular leak in patients with dengue.

Other mediators of vascular leak

Nitric oxide produced by the activated endothelium has been shown to modulate vascular permeability.¹⁰⁷ The presence of endothelium-derived nitric oxide was found to be higher in patients with DHF in the febrile phase, when compared with those with DF, although no changes were observed when these patients were followed longitudinally during illness.¹⁰⁸ In another study, it was also shown that platelet-derived nitric oxide was elevated in patients with DHF, and it was suggested that this led to vascular leak and haemorrhagic manifestations.¹⁰⁹ However, the activity of nitric oxide appears to be crucial for antiviral cytokines to exert their effects, as nitric oxide synthase-2 knockout mice, had increased viral loads, more severe disease and lethality when infected with the DENV.¹¹⁰ As nitric oxide also appears to play a protective role in acute dengue viral infection, its role in causing possible disease pathogenesis should be further investigated.

Other mediators that are known to cause vascular leak include bradykinins, complement proteins C3a and C5a, IL-33, fibrin products, prostaglandins E₂, F_{2a} and D₂.^{111–113} Patients with DHF were shown to have higher levels of C3a, C4a and C5a when compared with patients with DF, along with higher levels of factor D and lower levels of factor H.¹¹⁴ As the levels of C1q were normal in patients with DF and DHF, it has been suggested that a dysregulation of the alternative pathway of complement activation could contribute to complement activation.¹¹⁴ However, the role of complement components in mediating vascular leak in acute dengue has not been extensively studied and therefore, it is difficult to currently conclude on their role in increasing vascular permeability. However, complement components of both classical and alternative pathways have also been shown to cause liver damage in autopsies of children who died of DHF, suggesting that activation of complement could be playing a role in dengue.⁸⁷ We have evaluated IL-33 levels in patients with DHF and DF along with bradykinin at different phases of the illness. We did not observe any changes in either IL-33 or bradykinin levels in patients with acute dengue, during any of the phases when compared with healthy volunteers (Malavige, unpublished). The role of prostaglandins in acute dengue has not been extensively investigated so far and it would be interesting to evaluate whether these mediators also play a role in the disease pathogenesis.

Conclusions

Endothelial dysfunction leading to increased vascular permeability is a hallmark of severe dengue, leading to leakage of fluid into pleural and peritoneal cavities and shock. Although cytokines such as TNF- α , which are highly elevated in dengue, and are likely to result in increased vascular permeability, the roles of DENV-NS1 antigen and lipid mediators such as PAF in causing vascular leak are emerging. It may be that in practice, there are several pathways that co-contribute to vascular leak, but by understanding key mechanisms there may be opportunities for intervention. Therefore, although dengue vaccines that elicit neutralizing antibodies to DENV-NS1 are likely to be helpful in reducing disease pathogenesis due to NS1, drugs that block PAF receptors or the pathways in which PAF is generated may be helpful in the treatment of acute illness. However, there are many other mediators that cause vascular leak which have not been investigated in patients with dengue and it would be important to further evaluate their role to develop therapeutics for treatment of disease.

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Disclosures

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