HANS SHODH SUDHA , Vol. 1, Issue. 1, (2020) pp. 100-109

Unmasking host cell responses in severe Dengue pathogenesis

Riya Madan[1], Pooja Arora[2*]

Abstract:

Dengue virus infection, which is the cause of mortality across various regions of the globe, can be caused by any of the four recognized serotypes (DENV 1-4). The infection can range from dengue fever to severe and life-threatening dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). There has been a great deal of research to understand the pathogenesis of dengue and its severe forms. Multiple findings have revealed the role of various host's factors in abnormal homeostasis and increased vascular permeability. In order to control, cure and prevent the disease, it is vital to have a complete understanding of the entire mechanism of pathogenesis. In this review, we have attempted to elucidate the various roles of the host's immune system in pathogenesis of severe forms of dengue in a host.

Key words: dengue, hemorrhagic fever, pathogenesis, immune system

Introduction:

Dengue viral infection is regarded as the most prevalent and rapidly spreading mosquito-borne viral disease of human beings, spreading rapidly within countries and across the world (Guzman & Harris, 2015). The World Health Organization (WHO) report of 2017 estimates that 3.9 billion people in a total of 128 countries are at risk of dengue virus (DENV) infection per year (*Dengue and Severe Dengue*, n.d.). It is an arthropod-borne disease transmitted by *Aedes* mosquito and caused by the four dengue virus serotypes (DENV 1–4) which are genetically related and biologically similar.(Guzman & Harris, 2015)

Symptomatic dengue virus infection has been classified into dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) ("WHO | Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control. 2nd Edition. Geneva/: World Health Organization.," 2015) (Fig. 1). Dengue fever is characterized by the rapid onset of fever along with severe headache, retro-orbital pain, muscular pain (myalgia), joint pain (arthralgia), gastrointestinal discomfort, rash and possible minor hemorrhagic manifestations in the form of petechiae, epistaxis, and gingival bleeding whereas dengue haemorrhagic fever (DHF) is characterized by all the symptoms of dengue fever, in combination with hemorrhagic manifestations (positive tourniquet test or spontaneous bleeding), thrombocytopenia, and evidence of increased vascular permeability (Martina et al., 2009). On the other hand, dengue shock syndrome (DSS) is usually characterized by a rapid, weak pulse with narrowing of the pulse pressure or hypotension with cold, clammy skin and restlessness (*Dengue Haemorrhagic Fever*, n.d.). Dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) initially observed in Southeast Asia when children were found dying of an acute febrile disease accompanied by a complex of physiologic abnormalities affecting multiple organ systems has now spread throughout the world(Scott B. Halstead & Cohen, 2015).

According to an epidemiological and statistical study, the dengue incidence in India has sharply increased from 1998 to 2001 from 0.72 to 3.21 per million population. Over the period 1998–2009, 82 327 dengue cases (incidence: 6.34 per million population) were reported whereas during a more recent period

1. Department of Zoology, Hansraj College, University of Delhi, North Campus, Delhi-110007, India
2. *Corresponding author: Department of Zoology, Hansraj College, University of Delhi, North Campus, Delhi-110007, India, E-mail Address: pooja@hrc.du.ac.in

(2010–2014), 213 607 cases (incidence: 34.81 per million population) of dengue fever were observed. Since 2010, a dengue incidence of greater than 15 per million population has been reported annually (Mutheneni et al., 2017). Of the 50 million cases of dengue reported annually, it is estimated that almost 500,000 correspond to severe dengue, of which, over 20 000 individuals die because of the infection with this virus (Murray et al., 2013).

From the various studies made to understand the pathogenesis of dengue, it is clear that the interaction between the host immune system and virus replication plays an important role in the progression to severe disease such as DHF or DSS. Dengue Viral (DENV) proteins interact with the host cells to mediate viral replication and pathogenesis.

Various epidemiological and experimental evidences support the association between the severity of dengue illness and a previous dengue infection in consequence of preexisting enhancing antibodies (Tirado & Yoon, 2003). The activation of cells of both lymphoid (B and T cells) and myeloid origin (such as Monocytes, Macrophages) in response to the DENV infection can also lead to overproduction of pro-inflammatory cytokines which consequently cause the uncontrolled inflammatory response and the disruption of vascular epithelium integrity (Srikiatkhachorn et al., 2017b). The understanding of the pathogenesis of dengue severe forms is crucial clinically, immunologically and even epidemiologically. In this review, we aim to outline the role of host immune system in the progression of dengue to its severe forms such as dengue hemorrhagic fever in the host body.

Role of immune cells:

Monocytes: Monocytes are circulating blood leukocytes which play a crucial role in providing innate immunity against the pathogen through inflammatory response. These mononuclear phagocytic cells show plasticity to develop into macrophages or dendritic cells (Karlmark et al., 2012). Peripheral blood monocytes are a heterogeneous population of circulating leukocytes including CD14++ CD16" (classical), CD14++ CD16+ (intermediate), and CD14+ CD16++ (non-classical) monocytes, divided on the basis of expression of CD14 (membrane receptor for lipopolysaccharide) and CD16 receptors. These monocytes are further characterized and differentiated on the basis of expression of the chemokine receptors CCR2, CCR5 and CX3CR1 (Geissmann et al., 2003; Wong et al., 2012).

Monocytes have long been considered as the primary target of DENV infection and have been proven via several *in vitro* studies. The dramatic increase of DENV replication in monocytes and other Fc receptor bearing cells in the presence of specific antibodies against the virus is known as antibody dependent enhancement (ADE) of specific virus infection (Tirado & Yoon, 2003). Antibody Dependent Enhancement (ADE) of a virus infection occurs when preexisting antibodies against primary DENV infection binds to a DENV particle with a different serotype during subsequent infection. This complex is unable to neutralize the virus and gets attached to the Fcã receptors present on circulating Monocytes (and other Fcã receptors bearing cells) instead which, in turn, mediates the entry of DENV into the cell and leads to increase in the overall replication and production of the virus. According to a study analyzing infection and activation of human peripheral blood Monocytes by DENV through the mechanism of antibody-dependent enhancement, a >14 fold enhancement in secondary infection was observed with a heterotypic serotype while only a 6.6 fold increase in a homotypic serotype of DENV(Sun et al., 2011). Moreover, it was also observed that expression of CD86, CD32, CD14, CD11c, and DEN prM was significantly increased in peripheral blood mononuclear cells (PBMCs) from DHF patients as compared with DF patients corresponding to the implications for antibody dependent enhancement and immunopathogenesis of DHF (Durbin et al., 2008).

In response to the DENV infection, monocytes are triggered for the production of various chemokines and cytokines which are associated with severe dengue because of their role in increasing vascular leak and endothelial permeability (Castillo et al., 2019). These include high levels of TNF-á (Espina et al., 2003), IL-10 (Torrentes-Carvalho et al., 2009), IL-8, MCP-1, interferon ã-induced protein, (IP)-10, IL-6, IL-8, IL-10 and IL-1â (Green & Harris, 2014) (Fig. 2). The monocytes derived cells have also been shown to be responsible for the production of such cytokines and chemokines. The mode of action of these chemical factors has been discussed and illustrated shortly in the review.

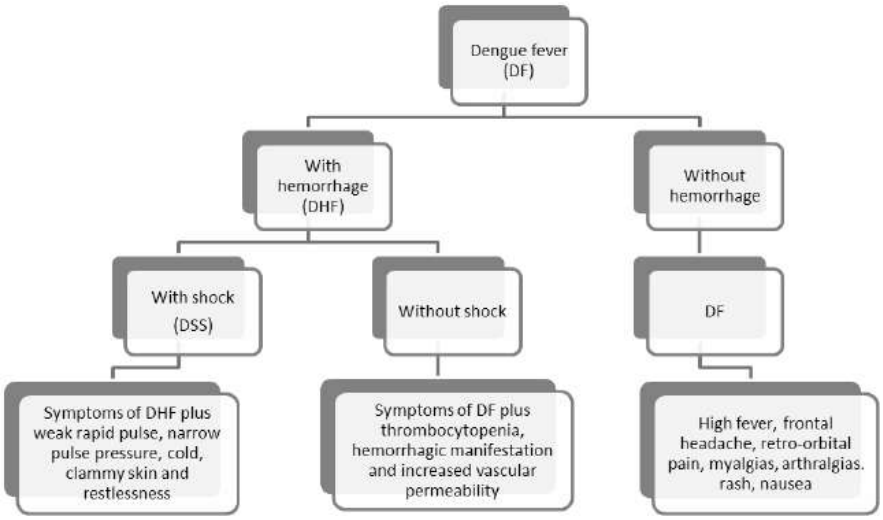

Figure 1: Classification of dengue fever on the basis of severity and clinical symptoms associated with them.

Macrophages: Macrophages are a diverse phenotype of professional phagocytic cells derived from bone-marrow precursors and parent monocytes in the peripheral blood. They are essential for the maintenance and defense of host tissues, doing so by sensing and engulfing particulate matter and, when necessary, initiating a pro-inflammatory response (Verschoor et al., 2012). Macrophages, along with monocytes are the prime targets of DENV and like monocytes, macrophages are also comprised of wide variety of subpopulations (Verschoor et al., 2012). It was observed that the *in vitro* DENV infected-macrophages secreted multiple innate cytokines and chemokines, including tumor necrosis factor alpha, alpha interferon (IFN-á), interleukin-1â (IL-1â), IL-8, IL-12, MIP-1á, and RANTES (Chen & Wang, 2002) (Fig. 2). A study has shown human primary splenic macrophages to be the principal dengue-permissive cells in spleen instead of T and B cells contributing in the initial steps of immune enhancements that ultimately lead to severe forms of dengue in some individuals (Blackley et al., 2007). The mannose receptors of macrophages have been shown to bind to envelope glycoprotein of all the four serotypes of DENV and thus aiding in viral entry (Miller et al., 2008). A C-type lectin on macrophages named CLEC5A activates NLRP3 inflammasomes which are essential for IL-1â and IL-18 secretion (Wu et al., 2013).

The levels of interleukin-6, interleukin-8, interleukin-10, interleukin-18, tumour necrosis factor-á, transforming growth factor-â, and cytotoxic factor-2, released by macrophages, have been observed to markedly increase in the case of DHF grade IV patients and are thus correlated with the severity of the disease (Chaturvedi et al., 2006). The cytotoxic factors, produced by CD4+ T cells and cytotoxic factor-2 by H-2A+ macrophages increase capillary permeability and damage the blood-brain barrier indicating their role in pathogenicity of DHF. Cytotoxic factor/cytotoxic factor-2 induce macrophages to produce free radicals, nitrite, reactive oxygen and peroxynitrite. The free radicals, besides killing the target cells by apoptosis also directly upregulate production of proinflammatory cytokines by macrophages (Chaturvedi et al., 1987; Gulati et al., 1983).

Interaction of dengue virus with macrophages and endothelial cell can lead to hemorrhage development. The virus infects several cell types including endothelial cells and also induces production of chemokines that attract macrophages. Along with the stimulation of endothelial cells by DENV NS2B/3, the chemical factors cause the endothelium damage and increased vascular permeability which ultimately results in hemorrhage development (Lin et al., 2014; Wan et al., 2018).

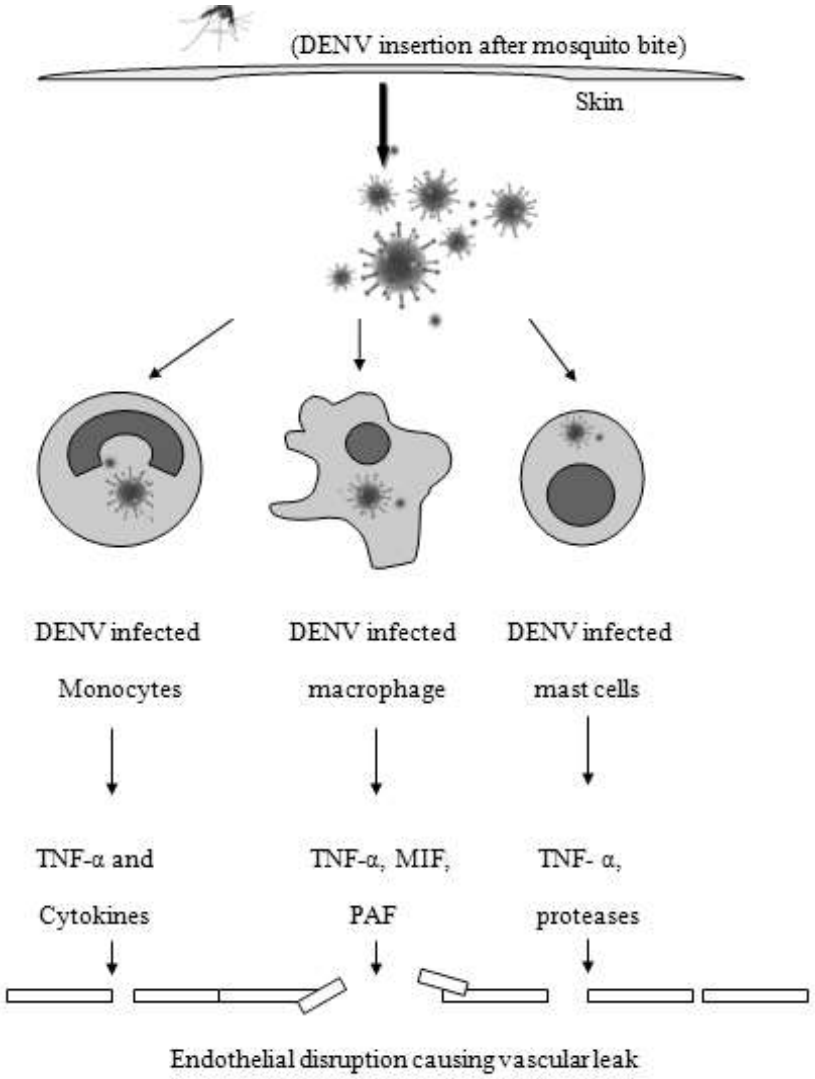

Figure 2: Role of monocytes, macrophages and mast cells in endothelial disruption causing vascular leak leading to severe forms of dengue infection.

Mast cells: In humans, mast cells develop from $CD34^{+}/CD117^{+}$ pluripotent progenitor cells originating in the bone marrow and are well known for their inflammatory roles during allergy (Gilfillan et al., 2011). Mast cells are the important cells of the immune system and are found in mucosal and epithelial tissues throughout the body. Two phenotypes of human mast cells are known: mucosal mast cells that produce only tryptase and connective tissue mast cells that produce chymase, tryptase, and carboxypeptidases (Irani et al., 1986; Krystel-Whittemore et al., 2016a). Similar to monocytes and macrophages, mast cells have also been reported to have a contribution to severe forms of dengue.

Human mast cells can express FCãRI and FCãRII receptors and are permissive to antibody-enhanced dengue virus infection, occurring in an FcãRII-dependent manner (Brown et al., 2006; Wan et al., 2018). ADE enhanced infection of mast cell results in infectious virus production and the induction of significant levels of cytokines, such as interleukin-1â (IL-1â), IL-6 and TNF-á, as well as chemokines CCL3, CCL4, and CCL5 (King et al., 2002).

DENV has been shown to accumulate in subcellular granules in mast cells after the infection and when these granules are released, infectious virus can be found inside and could be transported through the lymph system to other organs. After the activation of mast cells, there is a release of some potent immune mediators stored in granules which also include proteases such as chymase, tryptase, histamine, heparin along with various cytokines and chemokines.

The inflammatory stimuli released from mast cells such as VEGF (vascular endothelial growth factor), tryptase, histamine, chymase and TNF-á are known to be responsible for vascular leakage by disrupting the cell-cell junction (Fig. 2). The mast cells derived platelet activation factor is also associated with increased vascular permeability during the course of developing DHF (Avirutnan et al., 2013; Krystel-Whittemore et al., 2016b).

T-Lymphocytes: Besides the role of antibodies in the development of severe dengue, other adaptive immune components, such as T cells, have also been implicated in the pathogenesis. Without ignoring the role of the polyfunctional dengue-specific T cell response in the protection against DENV infection (Elong Ngono et al., 2016), it has been noted in some patients with secondary dengue infections, that when memory CD8+ T lymphocyte clones specific of previous infecting serotypes are activated, they have a low affinity for the new infecting serotype (S. B. Halstead et al., 1983). A study corresponding to virus-specific T cells in Thai children demonstrated that many dengue-specific T-cell cells showed higher affinity for previously encountered strains but were of low affinity for the infective virus; contributing to delayed viral elimination and systemic disturbances leading to severe forms of dengue (Mongkolsapaya et al., 2003).

In a secondary DENV infection, T cells are highly activated and produce multiple kinds of lymphokines whose levels are found to be significantly higher in DHF than DF. IFN-ã, IL-2 and TNF-á are mainly produced by DENV-specific T-cells upon activation and play a key role in pathogenesis of dengue and disease severity (Kurane et al., 2011).

Soluble factors:

Elevated levels of cytokines have been detected in DHF/DSS patients and may play a causal role in the vascular leak and coagulopathy syndromes characteristic of severe dengue (Bethell et al., 1998; Meena et al., 2019; Mustafa et al., 2001; Srikiatkhachorn et al., 2017a; Suharti et al., 2002; Tauseef et al., 2016).

· **TNF-á: I**t is a pro inflammatory cytokine produced by monocytes/macrophages, endothelial cells or adaptive immune cells namely CD4+ or CD8+ T cells. It is toxic to vascular endothelial cells and responsible for increased permeability (Espina et al., 2003; Yamaoka et al., 2002). It is known to induce reactive oxygen and nitrogen intermediates and signal for apoptotic cell death, increasing vascular permeability and ultimately leading to hemorrhage (Masood et al., 2018; Vitarana et al., 1991). A recent study by Meena et al. has found that an increase of one unit of TNF-*á* was associated with a decrease of 160 units of blood platelets; suggesting the role in pathogenesis of dengue (Meena et al., 2019). TNF-á works synergistically with IFN-ã and is known to mediate activation-induced death of T cells (Yamaoka et al., 2002). On the other hand, studies have also shown that TNF-á is responsible for induction of IL-6, the high levels of which have also been associated with dengue severity (Juffrie et al., 2001).

· **Vascular endothelial growth factor A (VEGF-A):** VEGF-A, is a 38 kDa heparin-binding, dimeric, disulfide-bonded glycoprotein (Senger et al., 1993). Tseng et al. have demonstrated elevated levels of VEGF in patients with dengue hemorrhagic fever and correlated it with D dimer level (Tseng et al., 2005). A study revealed that dengue virus can lower the production of soluble VEGF2 receptors by endothelial cells while increasing the expression of membrane-bound VEGF2. This combination will lead to increased biologically active free VEGF and increased VEGF2 receptor responsiveness that result in increased vascular permeability and plasma leakage (Srikiatkhachorn et al., 2007). VEGF is also associated with facilitation of coagulation–fibrinolysis pathway by induction of tissue factor (TF) expression in mononuclear and endothelial cells (Martina et al., 2009).

· **Platelet-activating factor (PAF):** Studies have shown significantly elevated levels of Platelet-activating factor (PAF) in patients with DHF (Jeewandara et al., 2015). DENV infected cells activate nuclear factor-8 b which further leads to production of PAF. On the other hand, PAF is also known to activate NF-*ê*B, which, in turn, regulates the production of several inflammatory cytokines such as TNF-á and IL-1â, ultimately leading to more PAF production (Im et al., 1997). Activation of platelet-activating factor receptor (PAFR) on endothelial cells and leukocytes induces increased vascular permeability, hypotension, and production of cytokines (Souza et al., 2009). Endothelial permeability is known to be disrupted by reduction in the expression of tight junction protein ZO-1 (Tornavaca et al., 2015). LPS present in blood is also known to amplify the production of PAF. Sphingosine-1-phosphate, a signaling phospholipid, is known to oppose the effects

of VEGF and a study has found significantly lower levels of S1P in blood of patients with DHF (Gomes et al., 2014). Various phospholipases have also been shown to act on the phospholipids to release PAF from cells such as monocytes, macrophages, mast cells, etc (Jeewandara et al., 2016). A recent investigation has shown that the mast cell activation leads to an elevated levels of secretory phospholipase A2s and thus increased range of PAF in DHF patients (Jeewandara et al., 2016).

· **Interleukins:** Higher plasma levels of IL-1â, IL-1Ra (Suharti et al., 2002), IL-6 (Juffrie et al., 2001), IL-13, IL-18 (Mustafa et al., 2001), IL-8 (Raghupathy et al., 1998), and IL-10 (Tauseef et al., 2016) have been found in patients with severe DENV infections. IL-2, IL-6 and IL-8 are known to be potent vascular permeability enhancing cytokines (Her et al., 2017). Platelets are known to form platelet–monocyte aggregates and interactions within them lead to production of cytokines such as IL-1*â*, IL-8 and IL-10 by Monocytes and thus this aggregate is, correlated with the presence of thrombocytopenia, and vascular leak (Jeewandara et al., 2015). Mildly higher IL-1*â* has been demonstrated in patients with severe dengue and is thought to associate with activation of fibrinolysis (Suharti et al., 2002) and increase in vascular permeability (Castro et al., 2011). A recent study indicated that IL-33, a pleiotropic pro-inflammatory cytokine, plays a 'disease-exacerbating' role in dengue infection and is probably driven by CXCR2-expressing cells (Marques et al., 2018).

· Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) and their endothelial tyrosine kinase receptor Tie-2 form a central signaling system in endothelial permeability (Gavin Thurston & Daly, 2012). Angiopoietin-1–mediated Tie2 activation maintains the quiescent state of the endothelium by stabilizing endothelial cell-cell junctions and by countering the permeabilizing effects of VEGF (G. Thurston et al., 2000). On the other hand, Angiopoietin-2 antagonizes the effects of Ang-1; it destabilizes the endothelium by disrupting cell-cell adhesion and primes the endothelial cells to the effects of pro-inflammatory cytokines and VEGF (Fiedler et al., 2006) . A study shows that DHF/DSS is associated with reduced Ang-1 plasma levels and increased Ang-2 levels. The two angiopoietins were shown to induce PAF very rapidly in bovine endothelial cell lines in a bi-phasic manner (Michels et al., 2012).

· Apart from these, interleukin 1 receptor antagonist, interferon ã –" lü "inducible protein 10, hepatocyte growth factor, soluble p75 tumor necrosis factor á receptor, vascular cell adhesion molecule 1, and matrix metalloproteinase 2 have also been significantly associated with significant plasma leakage (Her et al., 2017). Extremely high expression levels of monocyte chemoattractant protein-1 (MCP-1) were found in the plasma of DHF patients in a study by Lee et. al. their partial contribution to increased permeability and disrupted tight junctions of human vascular endothelium was also signified (Lee et al., 2006). On the other hand, DENV-triggered macrophage migration inhibitory factor (MIF) secretion can not only facilitate DENV replication through the regulation of autophagy but also worsen the severity of vascular leak by enhancing endothelial permeability (Lai et al., 2020).

Conclusion:

Endothelial dysfunction, increased vascular permeability and coagulopathy along with plasma leakage are the fundamental attributes in severe form of dengue DHF/DSS. Various studies have indicated the role of host immune system in pathogenesis of different forms of dengue infection. Both innate and adaptive immune responses lead to a cytokine storm which eventually causes alteration in the endothelial permeability. The interplay of immune cells such as monocytes, macrophages, mast cells, T cells and that of various soluble factors in pathogenesis of severe forms of dengue have been elucidated in this review. It is extremely crucial to have an integrated understanding of the mechanisms underlying the pathology of the disease and the contribution of immune system of host itself in its severity; in order to evaluate their role to develop therapeutics for treatment of disease.

References:

1. Avirutnan, P., & Matangkasombut, P. (2013). Unmasking the role of mast cells in dengue. *ELife*, *2013*(2). https://doi.org/10.7554/eLife.00767
2. Bethell, D. B., Flobbe, K., Cao, X. T., Day, N. P., Pham, T. P., Buurman, W. A., Cardosa, M. J., White, N. J., & Kwiatkowski, D. (1998). Pathophysiologic and prognostic role of cytokines in dengue hemorrhagic fever. *The Journal of Infectious Diseases*, *177*(3), 778–782. https://doi.org/10.1086/517807
3. Blackley, S., Kou, Z., Chen, H., Quinn, M., Rose, R. C., Schlesinger, J. J., Coppage, M., & Jin, X. (2007). Primary Human Splenic Macrophages, but Not T or B Cells, Are the Principal Target Cells for Dengue Virus Infection In Vitro. *Journal of Virology*, *81*(24), 13325–13334.

https://doi.org/10.1128/jvi.01568-07
4. Brown, M. G., King, C. A., Sherren, C., Marshall, J. S., & Anderson, R. (2006). A dominant role for FcãRII in antibody-enhanced dengue virus infection of human mast cells and associated CCL5 release. *Journal of Leukocyte Biology*, *80*(6), 1242–1250. https://doi.org/10.1189/jlb.0805441
5. Castillo, J. A., Naranjo, J. S., Rojas, M., Castaño, D., & Velilla, P. A. (2019). Role of Monocytes in the Pathogenesis of Dengue. In *Archivum Immunologiae et Therapiae Experimentalis* (Vol. 67, Issue 1, pp. 27–40). Birkhauser Verlag AG. https://doi.org/10.1007/s00005-018-0525-7
6. Castro, J. E. Z., Vado-Solis, I., Perez-Osorio, C., & Fredeking, T. M. (2011). Modulation of cytokine and cytokine receptor/antagonist by treatment with doxycycline and tetracycline in patients with dengue fever. *Clinical & Developmental Immunology*, *2011*, 370872. https://doi.org/10.1155/2011/370872
7. Chaturvedi, U. C., Nagar, R., Gulati, L., & Mathur, A. (1987). Variable effects of dengue virus-induced cytotoxic factors on different subpopulations of macrophages.*Immunology*, *61*(3), 297–301.
8. Chaturvedi, U. C., Nagar, R., & Shrivastava, R. (2006). Macrophage & dengue virus: Friend or foe? In *Review Article Indian J Med Res* (Vol. 124).
9. Chen, Y.-C., & Wang, S.-Y. (2002). Activation of Terminally Differentiated Human Monocytes/Macrophages by Dengue Virus: Productive Infection, Hierarchical Production of Innate Cytokines and Chemokines, and the Synergistic Effect of Lipopolysaccharide. *Journal of Virology*, *76*(19), 9877–9887. https://doi.org/10.1128/jvi.76.19.9877-9887.2002
10. *Dengue and severe dengue*. (n.d.). Retrieved June 20, 2020, from https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
11. *Dengue haemorrhagic fever*. (n.d.).
12. Durbin, A. P., Vargas, M. J., Wanionek, K., Hammond, S. N., Gordon, A., Rocha, C., Balmaseda, A., & Harris, E. (2008). Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. *Virology*, *376*(2), 429–435. https://doi.org/10.1016/j.virol.2008.03.028
13. Elong Ngono, A., Chen, H. W., Tang, W. W., Joo, Y., King, K., Weiskopf, D., Sidney, J., Sette, A., & Shresta, S. (2016). Protective Role of Cross-Reactive CD8 T Cells Against Dengue Virus Infection. *EBioMedicine*, *13*, 284–293. https://doi.org/10.1016/j.ebiom.2016.10.006
14. Espina, L. M., Valero, N. J., Hernández, J. M., & Mosquera, J. A. (2003). Increased apoptosis and expression of tumor necrosis factor-á caused by infection of cultured human monocytes with dengue virus. *American Journal of Tropical Medicine and Hygiene*, *68*(1), 48–53. https://doi.org/10.4269/ajtmh.2003.68.48
15. Fiedler, U., Reiss, Y., Scharpfenecker, M., Grunow, V., Koidl, S., Thurston, G., Gale, N. W., Witzenrath, M., Rosseau, S., Suttorp, N., Sobke, A., Herrmann, M., Preissner, K. T., Vajkoczy, P., & Augustin, H. G. (2006). Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. *Nature Medicine*, *12*(2), 235–239. https://doi.org/10.1038/nm1351
16. Geissmann, F., Jung, S., & Littman, D. R. (2003). Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity*, *19*(1), 71–82. https://doi.org/10.1016/S1074-7613(03)00174-2
17. Gilfillan, A. M., Austin, S. J., & Metcalfe, D. D. (2011). Mast cell biology: Introduction and overview. In *Advances in Experimental Medicine and Biology* (Vol. 716, pp. 2–12). NIH Public Access. https://doi.org/10.1007/978-1-4419-9533-9_1
18. Gomes, L., Fernando, S., Fernando, R. H., Wickramasinghe, N., Shyamali, N. L. A., Ogg, G. S., & Malavige, G. N. (2014). Sphingosine 1-Phosphate in Acute Dengue Infection. *PLoS ONE*, *9*(11). https://doi.org/10.1371/journal.pone. 0113394
19. Green, A. M., & Harris, E. (2014). Monocyte-plasmablast crosstalk during dengue. *Cell Host & Microbe*, *16*(1), 7–9. https://doi.org/10.1016/j.chom.2014.06.011
20. Gulati, L., Chaturvedi, U. C., & Mathur, A. (1983). Dengue virus-induced cytotoxic factor induces macrophages to produce a cytotoxin. *Immunology*, *49*(1), 121–130.
21. Guzman, M. G., & Harris, E. (2015). Dengue. *The Lancet*, *385*(9966), 453–465. https://doi.org/10.1016/S0140-6736(14)60572-9
22. Halstead, S. B., Rohanasuphot, S., & Sangkawibha, N. (1983). Original antigenic sin in dengue. *American Journal of Tropical*

Medicine and Hygiene, *32*(1), 154–156. https://doi.org/10.4269/ajtmh.1983.32.154

23. Halstead, Scott B., & Cohen, S. N. (2015). Dengue Hemorrhagic Fever at 60 Years: Early Evolution of Concepts of Causation and Treatment. *Microbiology and Molecular Biology Reviews*, *79*(3), 281–291. https://doi.org/10.1128/mmbr.00009-15
24. Her, Z., Kam, Y.-W., Gan, V. C., Lee, B., Thein, T.-L., Tan, J. J. L., Lee, L. K., Fink, K., Lye, D. C., Rénia, L., Leo, Y.-S., & Ng, L. F. P. (2017). Severity of Plasma Leakage Is Associated With High Levels of Interferon ã •"h-ü"Inducible Protein 10, Hepatocyte Growth Factor, Matrix Metalloproteinase 2 (MMP-2), and MMP-9 During Dengue Virus Infection. *The Journal of Infectious Diseases*, *215*(1), 42–51. https://doi.org/10.1093/infdis/jiw494
25. Im, S. Y., Han, S. J., Ko, H. M., Choi, J. H., Chun, S. B., Lee, D. G., Ha, T. Y., & Lee, H. K. (1997). Involvement of nuclear factor-kappa B in platelet-activating factor-mediated tumor necrosis factor-alpha expression. *European Journal of Immunology*, *27*(11), 2800–2804. https://doi.org/10.1002/eji.1830271109
26. Irani, A. A., Schechter, N. M., Craig, S. S., DeBlois, G., & Schwartz, L. B. (1986). Two types of human mast cells that have distinct neutral protease compositions.*Proceedings of the National Academy of Sciences of the United States of America*, *83*(12), 4464–4468. https://doi.org/10.1073/pnas.83.12.4464
27. Jeewandara, C., Gomes, L., Udari, S., Paranavitane, S. A., Shyamali, N. L. A., Ogg, G. S., & Malavige, G. N. (2016). Secretory phospholipase A2 in the pathogenesis of acute dengue infection. *Immunity, Inflammation and Disease*, *5*(1), 7–15. https://doi.org/10.1002/iid3.135
28. Jeewandara, C., Gomes, L., Wickramasinghe, N., Gutowska-Owsiak, D., Waithe, D., Paranavitane, S. A., Shyamali, N. L. A., Ogg, G. S., & Malavige, G. N. (2015). Platelet Activating Factor Contributes to Vascular Leak in Acute Dengue Infection. *PLoS Neglected Tropical Diseases*, *9*(2). https://doi.org/10.1371/journal.pntd.0003459
29. Juffrie, M., Meer, G. M., Hack, C. E., Haasnoot, K., Sutaryo, null, Veerman, A. J., & Thijs, L. G. (2001). Inflammatory mediators in dengue virus infection in children: Interleukin-6 and its relation to C-reactive protein and secretory phospholipase A2. *The American Journal of Tropical Medicine and Hygiene*, *65*(1), 70–75. https://doi.org/10.4269/ajtmh.2001.65.70
30. Karlmark, K., Tacke, F., & Dunay, I. (2012). Monocytes in health and disease—Minireview. *European Journal of Microbiology and Immunology*, *2*(2), 97–102. https://doi.org/10.1556/eujmi.2.2012.2.1
31. King, C. A., Anderson, R., & Marshall, J. S. (2002). Dengue Virus Selectively Induces Human Mast Cell Chemokine Production. *Journal of Virology*, *76*(16), 8408–8419. https://doi.org/10.1128/jvi.76.16.8408-8419.2002
32. Krystel-Whittemore, M., Dileepan, K. N., & Wood, J. G. (2016a). Mast cell: A multi-functional master cell. In *Frontiers in Immunology* (Vol. 6, Issue JAN). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2015.00620
33. Krystel-Whittemore, M., Dileepan, K. N., & Wood, J. G. (2016b). Mast cell: A multi-functional master cell. In *Frontiers in Immunology* (Vol. 6). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2015.00620
34. Kurane, I., Matsutani, T., Suzuki, R., Takasaki, T., Kalayanarooj, S., Green, S., Rothman, A. L., & Ennis, F. A. (2011). T-cell responses to dengue virus in humans. In *Tropical Medicine and Health* (Vol. 39). BioMed Central. https://doi.org/10.2149/tmh.2011-S09
35. Lai, Y.-C., Chao, C.-H., & Yeh, T.-M. (2020). Roles of Macrophage Migration Inhibitory Factor in Dengue Pathogenesis: From Pathogenic Factor to Therapeutic Target. *Microorganisms*, *8*(6), 891. https://doi.org/10.3390/microorganisms8060891
36. Lee, Y.-R., Liu, M.-T., Lei, H.-Y., Liu, C.-C., Wu, J.-M., Tung, Y.-C., Lin, Y.-S., Yeh, T.-M., Chen, S.-H., & Liu, H.-S. (2006). MCP-1, a highly expressed chemokine in dengue haemorrhagic fever/dengue shock syndrome patients, may cause permeability change, possibly through reduced tight junctions of vascular endothelium cells. *Journal of General Virology, 87*(12), 3623–3630. https://doi.org/10.1099/vir.0.82093-0
37. Lin, J.-C., Lin, S.-C., Chen, W.-Y., Yen, Y.-T., Lai, C.-W., Tao, M.-H., Lin, Y.-L., Miaw, S.-C., & Wu-Hsieh, B. A. (2014). Dengue Viral Protease Interaction with NF-êB Inhibitor á/â Results in Endothelial Cell Apoptosis and Hemorrhage Development. *The Journal of Immunology*, *193*(3), 1258–1267. https://doi.org/10.4049/jimmunol.1302675
38. Marques, R. E., Besnard, A.-G., Maillet, I.,

Fagundes, C. T., Souza, D. G., Ryffel, B., Teixeira, M. M., Liew, F. Y., & Guabiraba, R. (2018). Interleukin-33 contributes to disease severity in Dengue virus infection in mice. *Immunology*, *155*(4), 477–490. https://doi.org/10.1111/imm.12988

39. Martina, B. E. E., Koraka, P., & Osterhaus, A. D. M. E. (2009). Dengue virus pathogenesis: An integrated view. In *Clinical Microbiology Reviews* (Vol. 22, Issue 4, pp. 564–581). American Society for Microbiology Journals. https://doi.org/10.1128/CMR.00035-09
40. Masood, K. I., Jamil, B., Rahim, M., Islam, M., Farhan, M., & Hasan, Z. (2018). Role of TNF á, IL-6 and CXCL10 in Dengue disease severity. *Iranian Journal of Microbiology*, *10*(3), 202–207.
41. Meena, A. A., Murugesan, A., Sopnajothi, S., Yong, Y. K., Ganesh, P. S., Vimali, I. J., Vignesh, R., Elanchezhiyan, M., Kannan, M., Dash, A. P., & Shankar, E. M. (2019). Increase of Plasma TNF-á Is Associated with Decreased Levels of Blood Platelets in Clinical Dengue Infection. *Viral Immunology*, *33*(1), 54–60. https://doi.org/10.1089/vim.2019.0100
42. Michels, M., van der Ven, A. J. A. M., Djamiatun, K., Fijnheer, R., de Groot, P. G., Griffioen, A. W., Sebastian, S., Faradz, S. M. H., & de Mast, Q. (2012). Imbalance of Angiopoietin-1 and Angiopoetin-2 in Severe Dengue and Relationship with Thrombocytopenia, Endothelial Activation, and Vascular Stability. *The American Journal of Tropical Medicine and Hygiene*, *87*(5), 943–946. https://doi.org/10.4269/ajtmh.2012.12-0020
43. Miller, J. L., DeWet, B. J. M., Martinez-Pomares, L., Radcliffe, C. M., Dwek, R. A., Rudd, P. M., & Gordon, S. (2008). The mannose receptor mediates dengue virus infection of macrophages. *PLoS Pathogens*, *4*(2). https://doi.org/10.1371/journal.ppat.0040017
44. Mongkolsapaya, J., Dejnirattisai, W., Xu, X. N., Vasanawathana, S., Tangthawornchaikul, N., Chairunsri, A., Sawasdivorn, S., Duangchinda, T., Dong, T., Rowland-Jones, S., Yenchitsomanus, P. T., McMichael, A., Malasit, P., & Screaton, G. (2003). Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nature Medicine*, *9*(7), 921–927. https://doi.org/10.1038/nm887
45. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: Past, present and future prospects. *Clinical Epidemiology*, *5*, 299–309. https://doi.org/10.2147/CLEP.S34440
46. Mustafa, A. S., Elbishbishi, E. A., Agarwal, R., & Chaturvedi, U. C. (2001). Elevated levels of interleukin-13 and IL-18 in patients with dengue hemorrhagic fever. *FEMS Immunology and Medical Microbiology*, *30*(3), 229–233. https://doi.org/10.1111/j.1574-695X.2001.tb01575.x
47. Mutheneni, S. R., Morse, A. P., Caminade, C., & Upadhyayula, S. M. (2017). Dengue burden in India: Recent trends and importance of climatic parameters. *Emerging Microbes and Infections*, *6*(8). https://doi.org/10.1038/emi.2017.57
48. Raghupathy, R., Chaturvedi, U. C., Al-Sayer, H., Elbishbishi, E. A., Agarwal, R., Nagar, R., Kapoor, S., Misra, A., Mathur, A., Nusrat, H., Azizieh, F., Khan, M. A., & Mustafa, A. S. (1998). Elevated levels of IL-8 in dengue hemorrhagic fever. *Journal of Medical Virology*, *56*(3), 280–285. https://doi.org/10.1002/(sici)1096-9071(199811)56:3<280::aid-jmv18>3.0.co;2-i
49. Senger, D. R., Van De Water, L., Brown, L. F., Nagy, J. A., Yeo, K.-T., Yeo, T.-K., Berse, B., Jackman, R. W., Dvorak, A. M., & Dvorak, H. F. (1993). Vascular permeability factor (VPF, VEGF) in tumor biology. *Cancer and Metastasis Reviews*, *12*(3), 303–324. https://doi.org/10.1007/BF00665960
50. Souza, D. G., Fagundes, C. T., Sousa, L. P., Amaral, F. A., Souza, R. S., Souza, A. L., Kroon, E. G., Sachs, D., Cunha, F. Q., Bukin, E., Atrasheuskaya, A., Ignatyev, G., & Teixeira, M. M. (2009). Essential role of platelet-activating factor receptor in the pathogenesis of Dengue virus infection. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(33), 14138–14143. https://doi.org/10.1073/pnas.0906467106
51. Srikiatkhachorn, A., Mathew, A., & Rothman, A. L. (2017a). Immune-mediated cytokine storm and its role in severe dengue. In *Seminars in Immunopathology* (Vol. 39). Springer Verlag. https://doi.org/10.1007/s00281-017-0625-1
52. Srikiatkhachorn, A., Mathew, A., & Rothman, A. L. (2017b). Immune-mediated cytokine storm and its role in severe dengue. In *Seminars in Immunopathology* (Vol. 39, Issue 5, pp. 563–574). Springer Verlag. https://doi.org/10.1007/s00281-017-0625-1

53. Suharti, C., van Gorp, E. C. M., Setiati, T. E., Dolmans, W. M. V., Djokomoeljanto, R. J., Hack, C. E., ten, C. H., & van der Meer, J. W. M. (2002). The role of cytokines in activation of coagulation and fibrinolysis in dengue shock syndrome. *Thrombosis and Haemostasis*, *87*(1), 42–46.
54. Sun, P., Bauza, K., Pal, S., Liang, Z., Wu, S. jue, Beckett, C., Burgess, T., & Porter, K. (2011). Infection and activation of human peripheral blood monocytes by dengue viruses through the mechanism of antibody-dependent enhancement. *Virology*, *421*(2), 245–252. https://doi.org/10.1016/j.virol.2011.08.026
55. Tauseef, A., Umar, N., Sabir, S., Akmal, A., Sajjad, S., & Zulfiqar, S. (2016). Interleukin-10 as a Marker of Disease Progression in Dengue Hemorrhagic Fever. *Journal of the College of Physicians and Surgeons—Pakistan: JCPSP*, *26*(3), 187–190. https://doi.org/03.2016/JCPSP.187190
56. Thurston, G., Rudge, J. S., Ioffe, E., Zhou, H., Ross, L., Croll, S. D., Glazer, N., Holash, J., McDonald, D. M., & Yancopoulos, G. D. (2000). Angiopoietin-1 protects the adult vasculature against plasma leakage. *Nature Medicine*, *6*(4), 460–463. https://doi.org/10.1038/74725
57. Thurston, Gavin, & Daly, C. (2012). The Complex Role of Angiopoietin-2 in the Angiopoietin–Tie Signaling Pathway. *Cold Spring Harbor Perspectives in Medicine*, *2*(9). https://doi.org/10.1101/cshperspect.a006650
58. Tirado, S. M. C., & Yoon, K. J. (2003). Antibody-dependent enhancement of virus infection and disease. *Viral Immunology*, *16*(1), 69–86. https://doi.org/10.1089/088282403763635465
59. Tornavaca, O., Chia, M., Dufton, N., Almagro, L. O., Conway, D. E., Randi, A. M., Schwartz, M. A., Matter, K., & Balda, M. S. (2015). ZO-1 controls endothelial adherens junctions, cell–cell tension, angiogenesis, and barrier formation. *The Journal of Cell Biology*, *208*(6), 821–838. https://doi.org/10.1083/jcb.201404140
60. Torrentes-Carvalho, A., Azeredo, E. L., Reis, S. R. I., Miranda, A. S., Gandini, M., Barbosa, L. S., & Kubelka, C. F. (2009). Dengue-2 infection and the induction of apoptosis in human primary monocytes. *Memorias Do Instituto Oswaldo Cruz*, *104*(8), 1091–1099. https://doi.org/10.1590/S0074-02762009000800005
61. Tseng, C.-S., Lo, H.-W., Teng, H.-C., Lo, W.-C., & Ker, C.-G. (2005). Elevated levels of plasma VEGF in patients with dengue hemorrhagic fever. *FEMS Immunology and Medical Microbiology*, *43*(1), 99–102. https://doi.org/10.1016/j.femsim. 2004.10.004
62. Verschoor, C. P., Puchta, A., & Bowdish, D. M. E. (2012). The macrophage. *Methods in Molecular Biology*, *844*, 139–156. https://doi.org/10.1007/978-1-61779-527-5_10
63. Vitarana, T., de Silva, H., Withana, N., & Gunasekera, C. (1991). Elevated tumour necrosis factor in dengue fever and dengue haemorrhagic fever. *The Ceylon Medical Journal*, *36*(2), 63–65.
64. Wan, S. W., Wu-Hsieh, B. A., Lin, Y. S., Chen, W. Y., Huang, Y., & Anderson, R. (2018). The monocyte-macrophage-mast cell axis in dengue pathogenesis. In *Journal of Biomedical Science* (Vol. 25, Issue 1, pp. 1–10). BioMed Central Ltd. https://doi.org/10.1186/s12929-018-0482-9
65. WHO | Dengue haemorrhagic fever: Diagnosis, treatment, prevention and control. 2nd edition. Geneva: World Health Organization. (2015). *WHO*.
66. Wong, K. L., Yeap, W. H., Tai, J. J. Y., Ong, S. M., Dang, T. M., & Wong, S. C. (2012). The three human monocyte subsets: Implications for health and disease. *Immunologic Research*, *53*(1–3), 41–57. https://doi.org/10.1007/s12026-012-8297-3
67. Wu, M. F., Chen, S. T., Yang, A. H., Lin, W. W., Lin, Y. L., Chen, N. J., Tsai, I. S., Li, L., & Hsieh, S. L. (2013). CLEC5A is critical for dengue virus-induced inflammasome activation in human macrophages. *Blood*, *121*(1), 95–106. https://doi.org/10.1182/blood-2012-05-430090
68. Yamaoka, J., Kabashima, K., Kawanishi, M., Toda, K.-I., & Miyachi, Y. (2002). Cytotoxicity of IFN-ã and TNF-á for Vascular Endothelial Cell Is Mediated by Nitric Oxide. *Biochemical and Biophysical Research Communications*, *291*(4), 780–786. https://doi.org/10.1006/bbrc.2002.6487