Review Paper

The Pathogenesis and Immune-response in Dengue haemorrhagic fever

Abstract

Introduction

Dengue fever has spread to be endemic in addition of 100 countries to a total estimate incidence of 50 – 100 million cases annually globally. About 0.7% of these cases become the complication that is dengue hemorrhagic fever which is severe and leads to about 22,000 deaths annually.

The pathogenesis of benign dengue fever becoming dengue hemorrhagic fever, and aspects of the immune-response behind it, have remained relatively unknown.

Method

Existing literature on the Topic was retrieved through Google Scholar and PubMed searches, and the literature reviewed.

Results

Dengue hemorrhagic fever appears commoner in females and those with co-morbids such as diabetes-mellitus and obesity. Also, the case-fatality rate in severe dengue appears much bigger in females. The reasons for this are largely unknown but the additionally robust immune response in females, resulting in females to be

additionally prone to develop bigger inflammatory response or enhanced susceptibility to capillary permeability could be the reason.

It has been shown that viremic-load, including the initial viremic-load at the bite of the Aedes-mosquito may be a factor leading to dengue hemorrhagic fever.

Yet different factors felt to be causative in the pathogenesis of dengue hemorrhagic fever include the role of the viral-protein, and then that which is termed the original antigenic-sin, the antibody-directed enhancement, autoimmunity, inhibition of interferon-alpha and cytokine-storm within the memory-cells.

Regionally, certain different strains of the DENV also seem to be associated with dengue hemorrhagic fever.

Newer-vaccines, based on the immunology of the disease, offer much hope in the near future.

Conclusion

Much knowledge has been forthcoming in realizing the pathogenesis of dengue hemorrhagic fever. But, additional studies need to be done.

Key-words: dengue hemorrhagic fever; pathogenesis; co-morbids; capillary-permeability; autoimmunity;

INTRODUCTION

Dengue is considered the most problematic mosquito-transmitted viral disease in terms of morbidity and mortality. It is the most prevalent viral mosquito-borne disease, with over 2.5 billion humans at risk of exposure given its endemicity in more than 100 countries, compared to nine countries in 1970 [1–11].

Dengue fever is a benign, acute febrile syndrome found mainly in tropical regions. In a small proportion of cases, the virus causes increased vascular permeability that leads to a bleeding diathesis or disseminated intravascular coagulation (DIVC) known as dengue haemorrhagic fever (DHF) [3].

The WHO estimates that 50-100 million cases of dengue are incident annually, with approximately 500,000 of those cases (0.7%) resulting in dengue haemorrhagic-fever (DHF), with an estimated 22,000 deaths per year - mostly in children. In 20-30% of DHF cases, the patient develops shock, known as the dengue shock syndrome (DSS). [1, 4, 5 – 8]

The vectors are *Aedes aegypti*, which breeds in and around houses and buildings, and the smaller *Aedes albopictus* which breeds outdoors. They are day-biting mosquitoes, whose peak biting-hours are dawn, early morning and dusk [1–11]. Besides various different factors, the bite of the *A. aegypti* is more likely to cause severe dengue (DHF and dengue shock syndrome, DSS).This appears related to the

initial viral-load from the bite of the vector – the bite of the *A. aegypti* appears to cause a larger initial viral-load [12– 14].

Major sources of Aedes-breeding are at construction sites, solid-waste dumps, open-spaces and in factories [1–11].

METHODOLOGY

Existing literature on the Topic was retrieved through Google Scholar and PubMed searches, and the literature reviewed.

DISCUSSION

There are four (4) distinct, but closely-related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3 and DEN-4). Recovery from infection by one provides lifelong-immunity against that specific-serotype. Still, cross-immunity to the remaining serotypes after recovery is only partial and temporary. Subsequent infections by the remaining serotypes increase the risk of developing severe dengue [1–11].

Clinically, dengue fever can manifest as uncomplicated dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The mechanism behind the severe forms of dengue is the capillary leakage and increased vascular permeability of endothelial cells [1 – 11].

Severe dengue (DHF and DSS) may be commoner in females and those with co-morbids such as diabetes-mellitus and obesity. The case-fatality rate in severe dengue appears much higher in females [15 – 16].

The phases of dengue fever are febrile-phase, critical-phase and convalescence-phase.

Febrile-phase: The febrile-phase occurs 4-7 days after being bitten by an infected-mosquito and it lasts between 2 and 7 days. In this phase, there is high-fever (described as saddle-back fever), headache, retro-orbital pain, myalgia, typical macular-rash and facial-flushing [17 – 18]. There may be petechiae and minor gum bleeding, leucopaenia and thrombocytopenia. After about 3 to 5 days of fever, the patient enters into the critical-phase.

Critical-phase: Critical-phase is when the temperature settles (defer-vescence) typically during day 3 to 7 of dengue [17 – 19]. Plasma-leakage may be manifest during this period. In a patient who is not improving clinically despite the defervescence, beside a rising haematocrit and the presence of pleural effusion and ascites, it suggests plasma-leakage. Plasma-leakage must be detected early and treated to prevent it from progressing to severe decompensated and intractable shock - ultimately leading to multi-organ failure and death. Patients with coexisting conditions such as diabetes mellitus, pregnancy, obesity, renal-failure,

advanced age and chronic haemolytic-anaemia have a higher risk of dengue complications.

Whitehorn J et al (2000) showed that there was a dose-response relationship between the plasma viremia level and the proportion of mosquitoes with infectious saliva 14 days after blood-feeding. But, the likelihood of detecting infectious-saliva differed by mosquito-species and DENV-serotype. The detection of infectious-saliva was not as likely in blood-fed *A. albopictus*, compared with blood-fed *A. aegypti* [20].

By serotype, the odds of *A. albopictus* having infectious-saliva were significantly lower for blood-meals involving uptake of DENV-2 and DENV-4, compared with those involving uptake of DENV-1 or DENV-3. The authors' data identified the odds of *A. albopictus* becoming infectious as lower than the odds of *A. aegypti* becoming infectious after feeding on the blood of viremic patients [20].

Additionally, it was seen that plasma viremia-level as a risk factor for infectiousness among *A. aegypti* and *A. albopictus* [20].

Vaughn DW et al (2000) showed that higher peak-titers were associated with increased disease-severity. Increased dengue disease-severity correlated with high viremia-titer, secondary dengue virus-infection, and DEN-2 virus-type [21].

Sam SS (2013) showed that nine out of ten fatal-cases in a Malaysian hospital involved adult females. The mean age was 32, and not commoner in children as used to be thought. All had secondary dengue infection. The mean duration of illness prior to hospitalization was 4.7 days and deaths took place at an average of 2.4 days post-admission. Gastrointestinal pain, vomiting, diarrhea, intravascular-leakages and bleeding was seen in the majority of cases. DSS complicated with severe bleeding, multi-organ failure and coagulopathy were the primary causes of deaths [16].

Seven patients presented with thrombocytopenia and hypo-albuminemia, five of which had hemo-concentration and increased ALT and AST indicative of liver-damage. Co-morbidities, particularly diabetes mellitus, was common in the cohort. Prominent unusual presentations included acute renal failure, acute respiratory distress syndrome, myocarditis with pericarditis, and hemorrhages over the brain and heart. The majority of the patients presented with common clinical and laboratory warning-signs of severe dengue. Underlying co-morbidities appeared to contribute to the rapid clinical-deterioration in severe dengue [16].

Thus, there is a higher preponderance seen of fatal DHF/DSS amongst females. This is despite >55% of dengue cases seen at that hospital involved males. The observation was similar to that reported earlier where there was higher tendency of females to develop DHF/DSS [22 - 23] with higher mortality rate in

females [24] even though males consistently comprised the larger proportion of both DF and DHF, especially in the ≥15 years age group [24 - 25]. More deaths among girls, especially those among the pediatric group, was also reported in Vietnam in 1996–2009, despite the predominance of boys in dengue cases [26 - 27]. Currently, there is no satisfactory explanation for this phenomenon but there are suggestions that this may be due to the more robust immune response in females, resulting in females to be more prone to develop greater inflammatory response or higher susceptibility to capillary permeability [28 - 29]. An explanation need to be sought.

Thrombocytopenia, endothelial-activation, liver-dysfunction and disseminated intravascular coagulation (DIVC) associated with severe dengue, all contribute to bleeding in dengue. Minor bleeding like gum-bleeding and skin-petechiae are relatively common in dengue fever. These are usually self-limiting and insignificant. Gastrointestinal (GI) bleeding, menorrhagia, epistaxis and haemoptysis may be severe and require blood-transfusion. Frequently, the bleeding is occult.

Prolonged-shock and metabolic-acidosis further leads to severe bleeding and disseminated intravascular coagulation (DIVC).

Beside plasma-leakage and bleeding, dengue virus also ~~has~~ causes pathogenic-involvement of the liver. Elevations of liver-enzymes are usually mild and are common in dengue. Aspartate aminotransferase (AST) is usually more severely elevated compared to alanine aminotransferase (ALT). The relatively frequent isolation of dengue virus from liver-tissues of fatal-cases of dengue-hepatitis suggests that liver-injury is directly mediated by dengue virus infection of hepatocytes and Kupffer-cells [30]. Thus, liver-damage may also affect clotting-factors, in turn contributing to the bleeding.

Related to DENV infection of the liver, haemophagocytic lymphohistiocytosis (HLH) is rare but is a potentially fatal medical-condition that can happen after a patient has had severe dengue-infection. Haemophagocytic lymphohistiocytosis is characterized by hyperinflammation, uncontrolled-proliferation of activated-lymphocytes, prolonged fever, pancytopenia, jaundice and hepatosplenomegaly. But, dengue-associated HLH may be under-recognized due to overlapping signs and symptoms of HLH and dengue (e.g., fever, hepato-splenomegaly and pancytopenia). Identification of dengue-patients with, or at risk of developing, HLH is by detection of markedly-high serum-ferritin levels (≥ 500 mg/l) or serum IL2-receptor (≥ 2400 units/L). Signs and symptoms associated with HLH include hepatomegaly, splenomegaly and lymphadenopathy. Dengue with HLH usually has more prolonged-fever and hospitalization and is associated with warning-signs.

Laboratory findings include anemia, beside raised aspartate transaminase and alanine transaminase. Treatment includes high-dose corticosteroids and IVIG with or without etoposide (Meer Ahmad AM et al 2018).

Just recently, we have also seen quite a number of patients who present with progressive liver impairment accompanied by AST higher than ALT without plasma leakage. We thought that cytokine storm could have caused this even before full-blown haemophagocytosis, and we are still debating concerning when is the best time to initiate steroids in these patients (Leong CL, *ibid* Dec 2018)

Additionally, the heart, kidneys, lungs and central nervous system may ~~also~~ be involved both by direct action by the virus, beside plasma-leakage and bleeding in and around these organs.

One hypothesis concerning virus-virulence and the immune-enhancement hypothesis has been debated. Although dengue disease-severity has been associated with evidence of genetic-differences in dengue-strains, virus-virulence has been difficult to measure because of the lack of in-vivo and in-vitro models of the disease [31]. In addition to the outcome from virus-load and virus-variation, abnormal immune-responses of the host after dengue-virus infection may also account for the progression to DHF/DSS. Viral-autoimmunity is also involved in the pathogenesis of numerous similar viral-infections, such as human

immunodeficiency virus, human hepatitis C virus, human cytomegalovirus, herpes simplex virus and Epstein- Barr virus. Antibodies directed against dengue-virus non-structural protein 1 (NS1) shows cross-reactivity with human-platelets and endothelial-cells, which lead to platelet and endothelial-cell damage and inflammatory-activation. The hypothesis is that anti-DV (dengue virus) NS1 is involved in the pathogenesis of DHF/DSS [32]. There is also evidence that the presence of certain serotypes, including primary-infection with DENV-3 in the South-east Asia region and secondary-infection with DENV-2, DENV-3, and DENV-4 in the South-east Asia region, beside DENV-2 and DENV-3 from non-South-east Asia regions, increased the risk of severe dengue [33].

Immune-responses in dengue fever

Immune-responses to dengue-virus induces the production of all classes of antibodies, primarily targeting the virus envelope-proteins. The level of antibodies primarily depends on whether the individual has a primary or a secondary dengue-infection [34 - 35].

Fig. 1 shows the Timelines of Laboratory Changes viz-a-viz Clinical-features. In a primary-infection, low antibody-titres are observed - while in a secondary-infection, high IgG is detectable in the acute-phase which sometimes masks the detection of secondary-infection [36].

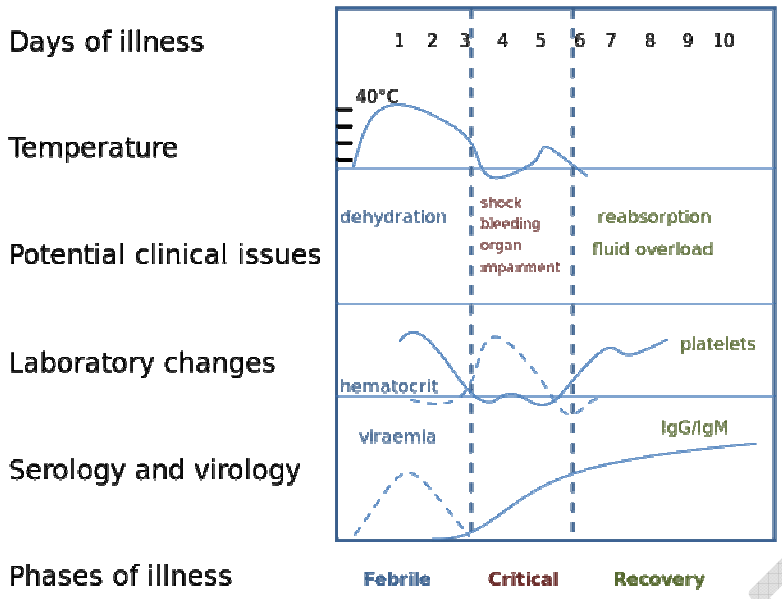

Fig 1. Timelines of Laboratory Changes viz-a-viz Clinical-features [36]

Antigen-detection is detectable through the incubation-period till the 12th Day (convalescent) of the illness. IgG and IgM start rising from the 2nd Day. IgM starts falling after the 10th to 11th Day – detectable window-period is found from 6th Day to about 30th Day. IgG becomes detectable from about 10th to 11th Day, and remains detectable, although peaking on about the 15th Day only. In secondary-infection, IgG is markedly elevated and remains elevated. IgM is not as markedly elevated as in primary-infection [36].

Primary Prevention of diseases classically comprises of Health Promotion and Specific Protection [37 - 41]. The best form of Specific Protection and which has been very successful in the Prevention and Control of previous vaccine-preventable infectious-diseases comprises of an appropriate Mass-vaccination

Program in the Endemic Areas. Vaccines are very much based on immunology, once again.

In late 2015 and early 2016, the first dengue-vaccine, Dengvaxia (CYD-TDV) by Sanofi Pasteur, was registered in several countries for use in individuals 9-45 years of age living in endemic areas. But overall, the much waited-for dengue-vaccine has been a disappointment both in its efficacy and its safety [42– 46]. If a sufficiently effective and safe vaccine can be found, it will transform dengue fever into a vaccine-preventable disease, and the disease can be quickly brought to near-eradication levels just like all of the previous vaccine-preventable diseases.

Takeda Pharmaceutical Company Limited, ("Takeda") in November 2017 announced the data from an 18-month interim analysis of the ongoing Phase 2 DEN-204 trial of its live, attenuated tetravalent dengue vaccine-candidate, TAK-003 (also referred to as TDV). This interim-analysis showed that children and adolescents who received TAK-003 had a relative-risk of symptomatic-dengue of 0.29 (95% CI: 0.13–0.72) compared to children and adolescents in the placebo control-group [47].

TAK-003 was found to be safe and well-tolerated in terms of solicited local-reactions and systemic adverse-events, relative to the placebo control-group. In participants who were sero-negative at baseline, a second-dose given at Month 3

improved the tetravalent sero-positivity rate at Month 6 to 86%, compared to 69% in the one-dose group. A booster dose at Month 12 resulted in a 100% tetravalent sero-positivity rate at Month 13 in participants who were sero-negative at baseline [47].

TAK-003 is currently under evaluation in the Tetravalent Immunization against Dengue Efficacy Study (TIDES), a large-scale Phase 3 efficacy-trial being conducted in eight dengue-endemic countries. Data from TIDES will be available in late 2018 [47].

The US National Institute of Allergy and Infectious Diseases (NIAID) has developed the LATV dengue vaccines TV003/TV005. A single-dose of either TV003 or TV005 induced sero-conversion to four DENV-serotypes in 74-92% (TV003) and 90% (TV005) of flavivirus-seronegative adults and elicited near-sterilizing immunity to a second dose of vaccine administered 6-12 months later [48–50].

The Phase III clinical-trial of the TV003 commenced in February 2016 among 17,000 volunteers in multiple locations in Brazil with the aim of determining its efficacy and safety. The estimated primary-completion date is June 2018, and the estimated study-completion date is December 2022 [48–50].

When vaccines are available which afford greater than 90% protection against all four strains, the risk of antibody-directed enhancement (ADE) in subsequent natural-infections, causing severe dengue, becomes remote because secondary infections would be rare. Dengue fever very likely will become reduced to sporadic-outbreaks of mostly the Sylvan-type, just like yellow-fever, once a successful mass-vaccination program of a safe and highly-effective vaccine becomes feasible and affordable (Meer Ahmad et al 2018).

Acquiring deeper insights into the different pathogenic mechanisms in the causation of dengue hemorrhagic fever will enable us in improving the different treatment strategies.

Several potential mechanisms have been identified which will be discussed here.

Fig 2. Potential mechanisms of DHF and DSS pathogenesis

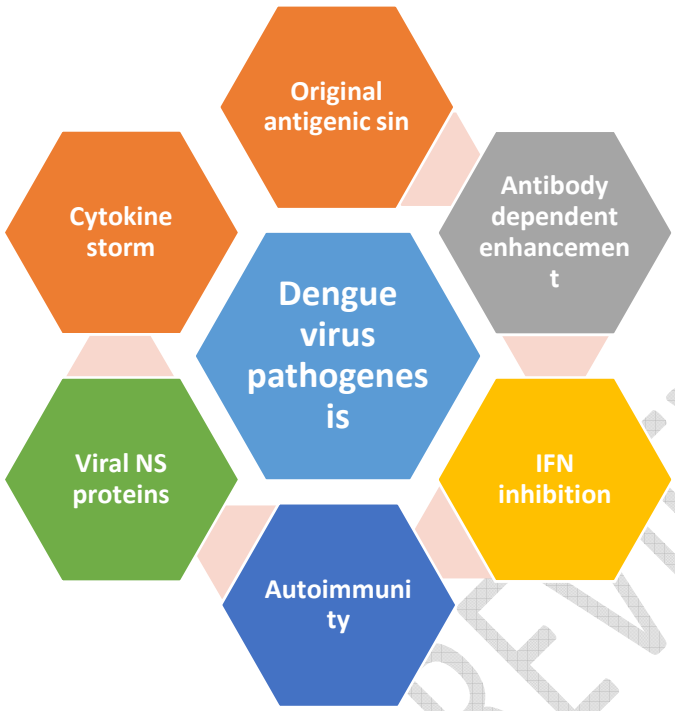

Table 1. Potential Pathogenic Mechanisms in the Causation of Dengue haemorrhagic fever

	Potential pathogenic mechanism	Descriptions
1.	Role of viral proteins	a. *Aedes egypti* transmitting DENV inoculates virus in the capillaries – the first targets are the dentritic-cells of the skin [51] where the virus enters the cells by receptor-mediated endocytosis [52 – 53], the DENV being composed of an electron-dense core made of the nucleo-capsid and single-stranded RNA; the core surrounded by lipid-bilayer of two transmembrane-glycoproteins [54]. b. Fusion of the viral and vesicular membranes releases nucleo-capsid

		with conversion of viral-proteins in the endoplastic-reticulum – the process releasing 10 proteins (3 structural and 7 non-structural,NS) – i.e capsid, prM and E structural beside NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5, non-structural) c. The NS-proteins suppress host immune-response, create environment for viral-replication, facilitate enzymatic-activities and re-structure cellular-membranes [55] d. CD 8+ T cells epitopes are mainly found in NS3 and NS5. In a contrast, CD4+ T lymphocyte-epitopes are found in the structural-proteins, the envelope and the capsid. e. Weiskopf *et al* demonstrated that live-attenuated tetravalent-vaccine opposed dengue-initiated CD8+ T cell responses against NS3 and NS5 [55].
2.	Original antigenic sin	a. When dengue-infection happens, the body's innate and adaptive immune-responses work together with B-cells producing antibodies that recognize and neutralize dengue viral-particles – T-cells recognizing and killing infected-cells and virus outside cells. b. Where the mechanism of dengue haemorrhagic fever is not clearly understood yet, Rothman et al describe the 'original antigenic sin' as the antibody-response to the secondary-infection that is influenced by the proliferation of cross-reacting memory-cells induced by the primary-infection [56].

		c. These memory-cells have a lower-threshold for activation of the immune-system than the naive-cells – and in this happening, there is a lower-affinity of binding with the infected-macrophages, not being as effective in clearing secondary-infections. d. Beside, memory-cells can recall a memory-response to different flavi-viruses such as Japanese encephalitis and yellow-fever – thus, augmenting the immune-pathology and damage to cells [56 – 57].
3.	Antibody-directed enhancement [57 – 59]	a. In secondary-infection, patients experience 'antibody-directed enhancement' (ADE) in which the body's immune-responbse actually makes the clinical-symptoms worse and increasing the risk of DHF [57]. b. There is an association between high viral-load and disease-severity which is attributed to ADE. c. With primary-infection by one DENV-serotype, primary-antibodies are produced conferring life-long immunity. When a new DENV-serotype subsequently infects, the disease tends towards severity with a high viremia. d. The antibody binds to the virus but is ineffective in neutralizing it in the reason of minor antigenic-differences between the serotypes. The immune-system is tricked – the virus continues to infect macrophages, multiplying and resulting in DHF with high amount of cytokines being released.
4.	Autoimmunity	a. Molecular structures are quite the

		same between platelets, endothelial-cells, coagulatory-proteins on one side and the viral-NS1, prM, and E-proteins that leads to cross-reactivity of the anti-NS1, anti-prM and anti-E with the host-proteins [60] b. Anti-platelet and anti-endothelial cell antibodies levels are found increased in DHF-patients than DF-patients. These antibodies are associated with thrombocytopenia and plasma-leakage from effect on endothelial-cells of micro-vasculature. c. Abnormal coagulopathy has been observed too in DHF which could be explained by the anti-NS1 and anti-E antibodies cross-reacting with blood-coagulation factors, fibrinogen and plasminogen [60 – 61].
5.	Interference with interferon-alpha	a. When DENV infects cells, IFNa/b is secreted by the T-cells to destroy the virus-infected cells. The IFNa/b binds to the infected-cells and activates the JAK/STAT pathway resulting in transcription and induction of numerous genes bringing about an anti-viral state [62]. b. Experimental-studies using mice find that pre-treatment with IFNa/b has protected from DENV-infection. But, IFNa/b does not inhibit replication after DENV-infection, meaning that IFN may not be active in secondary-infections. c. Jorge et al, in studying the role of the ten proteins coded by DEN-2 especially NS4B and NS4A in the inhibition of the IFNa/b response, showed that the DENV-pathogenicity increased with high-titres of IFNa/b

		[63]. d. A different study yet described the effect of NS2B on cGAS degradation which prevent sensing of mitochondrial-DNA of the virus-infected cells and subsequent inhibition of IFN [64]. e. Thus, viral NS4B and NS4A inhibit the IFN and result in high viremia in secondary-infections.
6.	Cytokine storm	a. In secondary-infections by a different strain, DHF is yet linked with high-levels of antibody-enhanced viral-replication early in disease, which is followed by a cascade of memory T-cell activation and a 'storm' of inflammatory-cytokines and various chemical-mediators. These compounds are released mainly from T-cells, monocytes/macrophages and endothelial cells and cause an increase in vascular-permeability [65]. b. St John et al demonstrated in a mouse-model that mast-cells are activated during DENV-infection and that mast-cell deficient mice had much reduced capillary-leakage [66]. Mast-cell derived mediators such as chymase are found increased in the blood in dengue-patients [67].

Additional clinical-studies are needed involving drug-probe studies against mast-cells to better fathom the contribution to disease.

The pathogenesis of severe dengue is a complex interplay between the viral-factors and proteins and the most intriguing immune-pathogenesis of dengue infection. There is still lots to learn about inducing immunity to DENV and developing targeted therapy. Focused research efforts will help to improve disease prevention and treatment in future.

Mangada MM and Rothman AL (2005) studied the interplay of different inflammatory-cytokines induced during a dengue-virus infection which appear to play a role in either protection or increased disease severity. The researchers measured the frequencies and characterized the cytokine-responses of DEN virus-specific memory CD4+ T cells in six volunteers who received experimental live-attenuated monovalent dengue-vaccines. IFN-gamma and TNF-alpha responses to inactivated dengue-virus antigens were detected in as much as 0.54 and 1.17% of total circulating CD4+ T cells, respectively. Antigens from the homologous-serotype elicited the highest IFN-gamma response. The ratio of TNF-alpha- to IFN-gamma-producing CD4+ T cells was found increased after stimulation with antigens from heterologous DENV-serotypes. Peptide-specific CD4+ T cell frequencies of up to 0.089% was detected by direct-staining using HLA class-II tetramers. IFN-gamma and TNF-alpha responses to individual HLA class II-restricted peptide-epitopes were detected in as much as 0.05 and 0.27% of CD4+ T cells, respectively. Peptide-sequences from the homologous-serotype elicited a

variety of cytokine-response patterns. TNF-alpha- to IFN-gamma-positive CD4+ T cell-ratios varied between peptides, but the ratio of the sum of responses was highest against heterologous-serotypes. The researchers conclude that the results demonstrate epitope sequence-specific differences in T-cell effector-function. These patterns of effector-responses may play a role in the immune-pathogenesis of dengue hemorrhagic fever [57].

CONCLUSION

Dengue fever has spread globally to be endemic in more than 100 countries to a total estimated incidence of 50 – 100 million cases annually globally. About 0.7% of these cases become the complication that is dengue hemorrhagic fever which is severe and leads to about 22,000 deaths annually.

The pathogenesis of benign dengue fever becoming dengue hemorrhagic fever, and aspects of the immunologic-response behind it, have remained relatively unknown.

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It has been shown that viremic-load, including the initial viremic-load at the bite of the Aedes-mosquito may be a factor leading to dengue hemorrhagic fever.

Additional factors felt to be involved in the pathogenesis of dengue hemorrhagic fever include the role of the viral-protein, and then that which is termed the original antigenic-sin, beside antibody-directed enhancement, autoimmunity, inhibition of interferon-alpha and cytokine-storm in the memory-cells.

Regionally, certain different strains of the DENV too seem to be associated with dengue hemorrhagic fever.

Vaccines against the disease are based on the immunologic-response. In this aspect, one recent vaccine has been a disappointment from the point of its efficacy and safety. Two more vaccines, conversely, offer much hope in the near future in both their efficacy and safety.

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