The Pathogenesis of Dengue

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1. Introduction

Dengue is a globally important arboviral infection transmitted by *Aedes* mosqitoes that endangers an estimated 2.5 billion people and represents a rapidly growing public health problem.[1] There are between 50 and 100 million infections each year, with approximately 500,000 cases admitted to hospital with severe and potentially life-threatening disease.[2-4] Dengue is an icosahedral, enveloped virus with a single-stranded positive sense genome; it is a member of the *flaviviridae* family and has 4 antigenically distinct serotypes (DENV1-4).[3, 5, 6] After infection of a susceptible host, an acute, self-limiting febrile systemic syndrome ensues. Resolution of infection occurs within 4-7 days and is associated with a robust innate and adaptive immune response. At present diagnosis is largely clinical, treatment is supportive and disease control is limited to tackling the vector.[1] Development of a vaccine would be a major advance in disease control but efforts have been hampered by the lack of an animal model of the disease and concerns about the role of the immune system in disease pathogenesis.[6]. The lack of an animal model further limits our understanding of immunopathogenesis.

Dengue is a syndrome and its pathogenesis an interplay between virus and host factors that remains incompletely understood [1, 7]. Explaining the heterogeneity between clinical presentations within a similar cross-section of the population remains a vital area of research. A better understanding of disease pathogenesis would greatly aid vaccine development by addressing specific concerns about vaccine safety and efficacy in light of the immune system's role in the development of clinical disease.[8] This review will examine the different components of our current understanding of dengue pathogenesis and then consider their implications for dengue vaccine development.

1.1 Clinical Signs, Symptoms and management

Clinically apparent dengue (DEN) virus infection is associated with a range of syndromes. Classical dengue fever is observed more frequently in adults and occurs after an incubation of 4 - 7 days.[9, 10] The clinical manifestations of children with DEN can differ from adults - cough, vomiting and abdominal pain appear to be more common.[11] The mortality rate in young children with DEN is significantly higher than in older children or adults.[12] The incidence of severe disease is perhaps highest in infants, which may reflect increased capillary fragility and lower compensatory reserve in this age group.[13] In classical DEN fever there is an abrupt onset of fever often associated with myalgia, headache and sometimes severe retro-orbital pain. Early in the illness the skin is flushed with petechiae appearing in the "critical" phase; a macular rash is observed in convalescence. The critical phase occurs around the time of defervescence, typically on days 3 to 7, and is associated with an increased propensity for capillary leakage and hemorrhage. In some individuals, capillary permeability manifests as a rise in haematocrit, pleural effusions or ascites.[14] This is the stage when life-threatening clinical complications such as circulatory shock are typically observed.[14] Disabling fatigue and depression may complicate recovery.[15] Thrombocytopenia is almost universal and minor bleeding may occur in mild infections - this can be severe in those with peptic ulcer disease.[16] Atypical clinical features of DEN are being more frequently reported but are probably still underappreciated.[17] These include encephalitis, myocarditis,

hepatitis, pancreatitis, retinitis and the acute respiratory distress syndrome (ARDS).[10, 18, 19] These atypical presentations likely reflect pathology at different endothelial surfaces.[17] Recognising the warning signs that may indicate progression to severe disease is essential for successful case management - these signs include abdominal pain, hepatomegaly, evidence of fluid accumulation and a rising haematocrit (or conversely a falling haematocrit suggestive of hemorrhage).[3] The current WHO guidelines recognize dengue as a clinical continuum from dengue to severe dengue.[3] Careful fluid resuscitation is life-saving in DEN.[3] Ringer's lactate has been shown to be efficacious in moderately severe DEN, and starch or dextran have been suggested for more severe cases.[20] Platelets are often given as prophylaxis to prevent haemorrhage; however this is a controversial area without a clear evidence base.[21, 22] In many settings DEN is diagnosed clinically; however the features of early infection are non-specific and mimic those of other febrile illnesses.[23-25] An early diagnosis would assist in patient triage and will have an increasingly important role as therapeutic drugs, e.g. anti-virals, become available for DEN.[26]

2. Immunopathogenesis of dengue

The severe phenotypes of dengue are observed not at the time when the viral burden is at its highest in vivo, but paradoxically when the virus is being rapidly cleared from host tissues by the innate and adaptive immune response. This has led to the suggestion that the pathogenesis of clinically important complications is closely linked to the host immune reponse.[27]

2.1 The humoral immune response and antibody-dependent enhancement

Although no correlate of immunity to dengue has been defined, it's hypothesized that the humoral immune response is vital for controlling DEN virus infection and for the expression of acquired immunity. In part, the belief that immunity to dengue is antibody-mediated stems from observations in other medically important Flavivirus infections, e.g. Japanese encephalitis virus and Yellow Fever, where the accepted vaccine-elicited correlate of immunity is virus-neutralizing antibody.[28-31] Confirmation that virus neutralizing antibody is a correlate of dengue immunity is most likely to come from expanded phase II and phase III trials of the Sanofi Pasteur developed ChimeriVax vaccine that are currently underway in Asia and Latin America.[32] (See Guy *et al.* article in this Special Issue).

Sabin demonstrated in the post-war era how infection with one serotype gave long-lasting protection to that specific serotype (homotypic immunity) and short-lived protection against the other serotypes (heterotypic immunity).[33] The basis for homotypic immunity is believed to be virus-neutralizing antibodies. The transient nature of heterotypic immunity is likely due to cross-reactive E-protein specific antibodies that when above a certain concentration threshold are protective. However, over time, these antibody concentrations decline and the individual is then susceptible to infection with other DEN virus serotypes.

Multiple prospective cohort studies in Asia and Latin America have identified secondary infection as an epidemiological risk factor for severe dengue.[34-38] Third, or even fourth infections also probably

occur in endemic settings, but hospital data and the age-related burden of dengue (children) suggests the vast majority of these tertiary or quaternary infections are clinically silent or very mild.[39] The leading explanation for increased risk of disease in secondary infection is that non-neutralising, cross-reactive antibodies elicited by a primary infection bind the virus which then have greater potential to infect Fc-receptor bearing cells. This phenomenon, called antibody-dependent enhancement (ADE), potentially increases the risks of developing severe disease by virtue of increasing the number of virus infected cells and therefore the viral biomass in vivo.[40, 41] Some evidence also suggests cells infected via an ADE process are immunologically modulated such that the local environment becomes more permissive for virus replication.[42] Cells of the monocyte-macrophage lineage in particular are believed to be a major site of dengue replication under conditions of ADE.[27, 43] The concept of ADE was initially proposed for dengue in 1977 and was supported by epidemiological observations in Cuba.[44, 45] Data from Cuba demonstrated that DHF was more frequently observed in those patients who had evidence of previous infection with a different serotype. Recent work has provided further support for the concept of ADE. This research has demonstrated that antibodies to dengue structural precursor-membrane protein (prM), a component of the humoral response to infection, are highly cross-reactive between DEN virus serotypes.[46] In vitro studies indicate that even when anti-prM antibodies are at high concentrations, they are non-neutralising but potently mediate ADE in Fc receptor bearing cells.[46, 47] The proposed basis for prM-mediated ADE is that on a proportion of virus particles prM is only partially cleaved from the virus surface during the virus maturation process. In this scenario, such "immature" virus particles that would otherwise be non- or less-infectious, are rendered infectious in an environment where anti-prM antibodies can mediate ADE.[46] These in vitro observations have led to the suggestion that a vaccine candidate should be designed in a way that would minimise the anti-prM response.[46]

A second epidemiological setting that implicates a role for ADE is primary infection of infants born to dengue-immune mothers. Primary infections in infants aged between 4-12 months of age can result in severe dengue, an outcome that is epidemiologically less common in younger infants and children aged between 1-2yrs of age. At the age of 3-4 months, maternally-derived virus neutralizing antibodies have generally declined below measurable levels in infants, however non-neutralising antibodies, which represent a much greater fraction of the virion-binding antibody population, remain present.[48-50] These virion-binding, non-neutralising antibodies are thought to enhance the risk of clinically apparent and severe dengue through a process of ADE.[51-54] In support of this hypothesis, neat plasma from 6-month old healthy Vietnamese infants enhances the infectivity of DENV-2 in Fc receptor bearing cells significantly more than plasma collected at the time of birth or at 1-yr of age.[52]

Concerns about ADE have led to fears that a vaccine could contribute to increased disease severity due to re-infection in the presence of incomplete protection to one or more serotypes. This hypothetical concern needs to addressed by careful, long-term follow-up of vaccine recipients. It is difficult however to envisage a scenario whereby vaccination actually increases the risk of dengue or severe dengue to a level where it exceeds the "natural history" of dengue epidemiology in endemic regions.

2.2 The cellular immune response

Cellular immune responses are also suggested to play a role in clearing virus infection and potentially triggering the development of severe disease. Activated memory T cells recognizing both conserved and altered peptide ligand epitopes, are suggested to be involved in the development of plasma leakage.[55] It is proposed that the expression of viral epitopes on the surface of infected cells trigger the proliferation of memory T cells and the production of pro-inflammatory cytokines that have an indirect effect on vascular endothelial cells resulting in plasma leak. The level of T cell response is thought to correlate to disease severity.[56, 57] Mongkolsapaya and colleagues in Thailand showed that T cells in severe infection have a relatively low affinity for the current infecting serotype but a high affinity for a past infection with a different serotype, i.e. they display characteristics of original antigenic sin.[57] Moreover, T cell responses in severe patients are mainly mono-functional in that they produce IFN-γ and/or TNF-α only and rarely CD107a, a marker of cytotoxic degranulation. Conversely, in patients with uncomplicated dengue, relatively more $CD8^+$ T cells expressed CD107a and only a few expressed only IFN-γ and/or TNF-α.[58] This is suggested to delay viral clearance, and via cytokine-mediated effects, potentially increase the risk of severe manifestations of disease. However, it remains to be shown, in even a temporal way, that T cells contribute to capillary leakage in vivo. Recent data in Vietnamese children suggests the emergence of activated T cells in blood of children with dengue is not synchronous with commencement of capillary permeability.[62] One possibility is that activated T cells are sequestered in tissues during acute dengue and are therefore difficult to detect at the time capillary permeability becomes apparent. The role of T regulatory cells is not clear in dengue. Their role in chronic infectious diseases has been studied but their role in acute viral infections is not well established.[63] However Luhn and colleagues demonstrated that they are functional and expand in acute dengue infection.[64] They may have a role in suppression of the production of vasoactive cytokines - perhaps the regulatory response is inadequate in severe disease?

The absence of good animal models of disease (as opposed to infection) is a hurdle to understanding the role of memory T cells in immunity and immunopathogenesis. For this reason, insights into the role of T cells in immunity will derive from prospective cohort studies, or vaccine trials.[65] A detailed commentary on the challenges of using cellular immune parameters as correlates of immunity to dengue has recently been published.[66]

2.3 Cytokines

It is thought that in some individuals with secondary infection, high viral burdens trigger expression of a wave of cytokine and other inflammatory molecules from innate and activated, cross-reactive T cells. This inflammatory mileau is hypothesized to mediate permeability in the vascular endothelium, allowing water and small molecules to leak from the intravascular space and in some cases leading to the severe manifestations of dengue.[67] Increased levels of many different cytokines have been observed in dengue infection.[68] In particular, higher concentrations of cytokines such as IFN-γ, TNF-α and IL-10 have been observed in the sera of patients with severe dengue in Vietnam, Cuba and India.[69-71]

Reduced levels of nitric oxide (NO) associated with increased levels of IL-10 have been described in patients with severe dengue.[72] NO contributes to immune regulation - lower levels of NO may result in the increased expression of pro-inflammatory cytokines.[73] In addition it is known that increased IL-10 levels correlate to reduced levels of platelets and reduced platelet function.[74] This phenomenon could, in part, contribute to the development of the bleeding complications observed in severe disease. Elevated levels of IL-6 have been observed in children with ascites.[75] TNF-α promotes increased endothelial permeability and it's plausible that increased levels of this cytokine could result in a more severe disease course.[76] It is likely that cytokines play a mutually synergistic role at the endothelial surface contributing to the development of the transient plasma leak, however the exact dynamic of this interaction has yet to be elucidated. It is worth noting that other infectious diseases and inflammatory disorders result in elevated cytokines without the attendant increased vascular permeability seen in severe dengue. Indeed, one of the challenges in dengue is to dissect those elements of the host immune response that are causally linked to capillary permeability from those that simply reflect the normal host immune response to a pathogen. This challenge is made more difficult by the absence of good animal models of disease.

2.4 Complement

Reduction in the levels of complement components have been described in patients with severe dengue, suggesting that complement activation may have a role in the pathogenesis of severe disease.[77, 78] In particular it has been suggested that excessive complement activation at endothelial surfaces contributes to the vascular leak observed in severe disease.[79] NS1, a non-structural viral protein that is secreted from infected cells and present in blood in concentrations exceeding 1μg/ml in some patients, could be an important modulator of the complement pathway. DEN virus NS1 attenuates classical and lectin pathway activation of complement by directly interacting with C4.[80] NS1 promotes efficient degradation of C4 to C4b and by this mechanism, NS1 is suggested to protect DENV from complement-dependent neutralization in solution.[80] The significance of these observations with the independent observation that early NS1 concentrations in blood are positively associated with disease severity are not yet clear, but are consistent with a role for NS1 and complement in pathogenesis .[81, 82] Finally, it is plausible that the low levels of complement observed in severe dengue are merely a marker of a severe systemic disease rather than an indicator of their role in capillary permeability.

2.5 Moving beyond descriptive studies in immunopathogenesis

Much of the literature describing the immunopathogenesis of dengue has been correlative in nature, e.g. temporal associations between elevated cytokine concentrations in the febrile phase of dengue is often interpreted as being causally linked to the important clinical events of plasma leakage or bleeding manifestations. The challenge in this area of research is to move beyond descriptive studies and begin to identify causal immunopathogenic mechanisms. Therapeutic randomized controlled intervention trials, with either anti-viral drugs or immunomodulatory agents e,g. corticosteroids, perhaps will offer insights into pathogenesis in a more direct fashion. Such trials are underway (ClinicalTrials.gov

identifier NCT01096576 and ISRCTN39575233). Alternatively, improvements in animal models of dengue have occurred such that a productive, sometimes fulminant, acute virus infection can be established.[83] As yet however, no small animal model mimics the virological and pathophysiological events that occur in a child with severe dengue, in particular the relatively slowly evolving capillary permeability that clinically manifests between day 3-6 of illness coupled with a fast declining viral burden. Clearly then there is scope for further research into animal models and strong justification for studying the host response during randomized controlled treatment trials in dengue patients.

3. Host determinants of disease severity

Only a small proportion of individuals with secondary DEN virus infections (and an even smaller proportion with primary infection) develop severe disease.[1] Therefore it follows that other variables, besides pre-exising immunity, shape the outcome of infection. This section will consider the role of age, gender, genetic susceptibility determinants and pre-existing medical conditions in determining the clinical phenotype.

3.1 Age and gender

Age is a risk factor for severe dengue and death. For example, the odds of a Vietnamese child 1-5yrs of age dying as an inpatient in a Ho Chi Minh City Hospital between 2001-2009 was four-fold higher than a child 11-15yrs of age. The greater relative prevalence of DSS in children relative to adults is likely tied to their having an intrinsically more permeable vascular endothelium, as demonstrated previously by Gamble et al in healthy Vietnamese children and adults.[84] The risk for more severe outcomes in young children argues for vaccination implementation strategies to include this most vulnerable population.

Gender is also a risk factor for severe dengue and death- females are over-represented in several case series of children with DSS.[85-87] Girls were also over-represented amongst severe (odd ratio 1.19) and fatal cases (odd ratio 1.57) in the cohort of dengue inpatients at three large hospitals in Ho Chi Minh City between 2001-2009.[85] The basis for an over-representation of females amongst severe and fatal cases has been suggested to be due to differences in health-care seeking behavior on behalf of girls and boys in Asian countries, i.e. girls may be presenting later in the course of their illness, leading to a higher likelihood of severe outcomes.[85] An alternative hypothesis is that physiological or immunological differences exist between males and females that explain these gender differences. Clearly, more research is required to understand the role of age and gender as determinants of outcome.

3.2 Genetic determinants of dengue susceptibility

Epidemiological studies suggest that people with African ancestry are less susceptible to the severe manifestations of dengue infection- the evidence for this is strongest in studies from Cuba and Haiti.[88, 89] Other evidence for a host genetic basis to susceptibility stems from case-control association studies.

In the immune response to DEN virus infection, viral antigens are presented to T cells in association with the major histocompatability complex (HLA) class I and II system. Some HLA alleles are suggested to be associated with different clinical phenotypes of dengue.[90] In addition, there is a suggestion of an association between HLA allele and susceptibility to specific dengue serotypes, for example DENV-1 and HLA *0207 and DENV-2 and HLA *B52.[91] In addition some associations appear to be protective, for example HLA DR alleles in Mexican and Vietnamese populations.[92, 93] However, most studies of genetic association with dengue are undermined by issues of sample size, multiple testing, unknown population stratification and variable case definitions. Indeed, with the exception of DC-SIGN, no association in an HLA allele or candidate gene has been replicated in an independent study.

Despite these limitations in study design, various single nucleotide polymorphisms (SNPs) have been associated with both protection and vulnerability to dengue infection.[90] The vitamin D receptor modulates the immune response by stimulating cell-mediated immunity and inhibiting lymphocyte proliferation; a polymorphism in the receptor appears to protect against severe dengue.[94] The Fcγ receptor mediates entry of antibody-coated DENV into target cells; the same study demonstrated that homozygotes for the arginine variant at position 131 of the Fcγ receptor gene may also be protected from severe disease.[94] This is perhaps because these homozygotes have less ability to opsonise IgG2 antibodies. There have been conflicting results from studies looking at polymorphisms in the TNF-α gene - data from Vietnam did not show an association, whereas investigators in Venezuela reported a significantly increased prevalence of the TNF-308A allele in patients with severe dengue.[94, 95] Dengue infection of dendritic cells is mediated by the attachment factor, dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN1, encoded by CD209). The G allele of the variant of the promoter region (DC-SIGN-336) was associated with protection against dengue fever, but not dengue haemorrhagic fever.[96] This finding suggests that different disease phenotypes may be partly related to the activity of CD209 and the degree of DC-SIGN expression. Another area of focus in determining susceptibility to dengue has been polymorphisms in human platelet antigens (HPA). HPAs mediate the interaction between platelets and endothelium. Data from an Indian cohort showed that HPA-1a and HPA-2b alleles were expressed more frequently in patients with severe disease.[97] In addition the study demonstrated that clinical shock was seen more frequently in HPA-1 heterozygotes. It is possible that cross-reactive antibodies formed in the context of dengue infection may bind to HPA leading to platelet reduction.

In the era of genome-wide association studies (GWAS), a more expansive approach to understanding genetic susceptibility to severe dengue is now possible and desirable. Equally, this approach could be used in a pharmacogenomics fashion to understand the genetic basis for adverse events, immunogenicity and durability of immune responses in participants of large dengue vaccine trials. This approach typically requires thousands of cases that fit a well-defined clinical outcome and population-matched controls. The strength of the approach is that it has power to detect relatively small contributions to genetic risk whilst also discounting any population stratification in cases or controls.

The next major advances in our understanding of host genetic susceptibility to severe dengue, or the immune response to vaccination, are likely to come from GWAS methods.

.3.3 Pre-existing comorbidities and predisposition to severe dengue

There have been few studies that have explored associations between underlying chronic diseases and predisposition to severe dengue. It was observed during the Cuban epidemics that chronic conditions, for example bronchial asthma, diabetes mellitus and sickle cell disease, were over-represented in patients with severe disease.[98-101] In addition it has been observed that a chronic medical condition is present in up to 70% of fatal cases in some case series of adults.[102] Data from Singapore suggests that co-morbidities such as hypertension are common in older patients with DEN, but infections in this age group are relatively benign.[103] Case control studies are necessary to further determine the role that chronic illness plays in predisposing the host to the severe manifestations of disease. Chronic hepatitis B virus infection is a common health problem in DEN-prevalent areas; co-infection does not appear to affect the clinical course but is associated with higher liver enzyme elevations.[104] DEN-HIV co-infection is an area of interest. There has been a recent intriguing observation that DEN NS5 protein inhibits HIV replication.[105] However a small case series in Singapore does not suggest the clinical course of dengue is any different in patients with HIV.[106] Pre-existing morbidities, more common in adults, could also be a determinant in the safety profile of live attenuated dengue vaccines. For example, it is unknown how HIV co-infection impacts the safety profile of live attenuated dengue vaccines currently in development. Similarly, it is unknown if secondary immunodeficiency (e.g. through long-term corticosteroid use) is a factor in the safety and reactogenicity of live attenuated dengue vaccines. Insights in these areas will accumulate as clinical development of candidate vaccines progresses, or alternatively, from post-marketing surveillance.

4. Thrombocytopenia and coagulopathy in dengue pathogenesis

4.1 Thrombocytopenia

As discussed above thrombocytopenia is an almost universal finding in dengue. This occurs as a result of both reduced production and increased destruction of platelets.[107-109] It has been suggested that the degree of thrombocytopenia correlates with clinical severity and complement activation.[110] As a result of reduced platelet counts and increased complement activation there is increased vascular fragility and thus an increased risk of haemorrhage.

4.2 Coagulopathy

Disordered coagulation, together with plasma leak and thrombocytopenia is likely to contribute to the haemorrhage observed in some cases of severe dengue.[111] This coagulopathy is likely to be mediated by cytokines. In a mouse model of the disease elevated levels of TNF-α correlated with endothelial cell dysfunction and haemorrhage.[112] Elevations of IL-6 and IL-8 have been associated with disordered coagulation and fibrinolysis in dengue.[109, 111] Data from children in Vietnam has shown that increased APTT and reduced fibrinogen are consistently observed in patients with dengue.[113] The same study

showed minor elevations in prothrombin time (PT). Infections normally result in increased fibrinogen, unless these conditions are complicated by disseminated intravascular coagulation (DIC). However the minor changes in PT together with the infrequent observation of fibrinogen degradation products (FDPs) do not support DIC being the cause of dengue-associated coagulopathy.[113] There are conflicting opinions about whether fibrinolysis or impairment of the fibrinolytic pathway occurs in dengue pathogenesis.[114] It is clear that the exact mechanisms contributing to dengue coagulopathy are incompletely understood and that a better understanding is needed to allow for improvements in supportive care.

5. Viral factors in pathogenesis

5.1 Virus epidemiology and virulence

There are 4 antigenically distinct serotypes of dengue virus - DENV-1, DENV-2, DENV-3 and DENV-4. Each of the DEN virus serotypes is capable of causing severe dengue and early viral burdens in the course of illness are associated with severe disease.[74, 82, 115] Oscillations in the prevalence of each serotype are common in endemic settings. Typically, one serotype is dominant for a period of 2-4 years, after which it declines in prevalence as a different serotype(s) emerges to replace it.[116, 117] The basis for the decline in prevalence of a serotype is presumably the accumulation of herd immunity such that the number of susceptible humans available is diminished. This cycling may also be due to immune enhancement when a new serotype is encountered. Each DENV serotype is as phylogenetically distinct from one other as the DENV group is from Japanese encephalitis virus, suggesting that each serotype could be considered a separate virus with distinct in vivo characteristics. This concept is now gaining wider recognition. For example, the kinetics of viremia and NS1 antigenemia in DENV-1 infections is distinctly different (higher) from DENV-2 infections in Vietnamese children.[52, 60] Similarly, Vietnamese infants with primary DENV-1 infections have significantly lower plasma NS1 concentrations than DENV-2 infected infants at the time of hospital presentation. Furthermore, a prospective study of hospitalized Thai children suggested DENV-2 and -3 were twice as likely to result in DHF as DENV-4.[118] Collectively, these data are examples that suggest each DEN virus serotype has its own constellation of virological characteristics in human hosts. Further research is needed to understand the breadth of these differences in humans, but also to understand differences with the mosquito host.

Adding to the complexity of DEN virus biology, within each DEN virus serotype there are distinct phylogenetic genotypes that appear to have differing geographical ranges. Increasingly, it is becoming clear that fitness differences exist between genotypes of the same serotype.[119] For example, there is evidence that the Asian/American subtype of DENV-2 is associated with more severe disease and has a fitness advantage in infected mosquitoes compared to the American genotype of DENV-2.[120] Conversely, in Viet Nam, the introduction of the Asian 1 genotype of DENV-2 led to the complete replacement of the resident Asian/American genotype of DENV-2.[117] The transmission fitness advantage of Asian 1 viruses was attributed to this virus attaining higher viremia levels in humans.[117]

Other examples of genotype replacement events have occurred in Sri Lanka and Thailand. In Sri Lanka, the introduction of a new genotype DENV-3 virus was associated with an increased incidence of severe dengue.[121] Recently, evidence of antigenic differences between genotypes of the same serotype has emerged.[122, 123] To date these differences have been elucidated using mAbs specific to the E protein and it remains to be seen whether such differences are present in the face of polyclonal immune sera. Prospective cohort studies that can follow the introduction of different DEN virus genotypes into the same population are needed to understand the clinical and epidemiological significance of antigenic variation between DEN virus genotypes. It is worth remembering however that clinically apparent recurrent infections caused by the same serotype of DENV virus have not been documented, suggesting antigenic variation between genotypes might not be clinically relevant. Nevertheless, this is an important question since any evidence of "immune escape" within a serotype would have implications for vaccine development.

5.2 Cellular and tissue targets of DEN virus infection

The very early events in DEN virus infection of the human host are poorly understood. After the bite of an infected mosquito it is thought that initially immature Langerhan's cells in the dermis are infected first.[124] These infected cells migrate to the lymph nodes, resulting in infection of cells of the macrophage-monocyte lineage. This amplifies the infection, which is then disseminated via the lymphatic and vascular system. After this primary viraemia circulating monocytes within the blood and macrophages within the liver, spleen and bone marrow are infected.[125, 126] The range of tissues and cell types infected with DEN viruses suggests that the host receptor(s) are broadly distributed. To date, there is evidence for several candidate host receptors, for example mannose binding protein, heparan sulphate, chondroitin sulphate and DC-SIGN.[127-129] The factors that determine the number of cells infected at specific sites may also influence the level of immune activation, and thus have a role in determining the disease phenotype.[111, 125] The time between the bite of an infected mosquito and the onset of clinical symptoms is believed to be 3-5 days [130, 131] Whether infected individuals in this pre-syndromic phase are capable of transmitting virus to a biting mosquito is unknown.

The study of viral tropism and in vivo pathology in dengue has been hampered by the lack of an animal model of disease and limited autopsy data from fatal cases. Nevertheless, post-mortem studies have detected suggested DEN viral antigen in a range of host cells and tissues.[132] There was also selective apoptosis of endothelial cells in the pulmonary and intestinal vasculature in one study, although this needs to qualified since differentiating true disease pathology from post-mortem tissue changes can be difficult.[133]

6. Conclusions

Dengue continues to be a growing public health threat in many parts of the world. It is clear that vector control is an inadequate public health tool in endemic areas. Whilst careful clinical management can reduce the case-fatality rate amongst hospitalized patients to less then 1%, the disease burden places enormous pressure on health services and has a substantial social and economic impact. [134] The virulence of the virus plus the flavivirus infection history, age, gender and genotype of the host all appear to be determinants of outcome. The host immune response appears to play a central role in pathogenesis and much new information on this topic has been derived in the last 10 years. Nonetheless, much work is still needed to identify the precise causal mechanisms of the dengue capillary permeability syndrome, and in turn, how this can be modulated to improve patient management. A better understanding of dengue immunopathogenesis will assist not only development of therapeutic interventions but also the understanding of dengue vaccine efficacy or vaccine adverse events.

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