Dengue

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Introduction: Dengue is vector-borne viral infection that endangers an estimated 2.5 billion people. Disease caused by dengue ranges from a relatively minor febrile illness to a life-threatening condition characterised by extensive capillary leak. A greater understanding of dengue has the potential to improve both the clinical management of individual cases and the control of the disease.

Sources of data: We searched the available literature suing PubMed, Embase and Web of Science for relevant articles and abstracts.

Areas of agreement: Addressing our gaps in the understanding of disease pathogenesis and improving our knowledge of dengue virus biology are necessary in order to develop tools to effectively control, diagnose and treat the disease.

Areas of controversy: The pathogenesis of dengue is multifactorial and depends on both host and virus factors. A more integrated understanding of disease pathogenesis is necessary.

Areas timely for developing research: There are many questions related to disease pathogenesis, development of diagnostics, drug and vaccine development and individual case management that need addressing if the disease is to be successfully tackled.

Keywords: Dengue / Dengue Haemorrhagic Fever / Dengue Shock Syndrome / pathogenesis / vaccine / drug development

Introduction

Dengue is a viral infection transmitted by *Aedes* mosquitoes.[1] Dengue is a *Flavivirus* with four different antigenically distinct serotypes (DENV1-4).[2, 3] It is a rapidly growing health problem with an estimated 2.5 billion people at risk, mainly in countries of south and south-east Asia, the Caribbean, Central and South America, and more recently in Africa.[4, 5] The spread of dengue is thought to be due to combination of factors: increased urbanisation, population growth, migration and international travel and the difficulties of effective vector control.[6] Climate change may be a contributing factor in the global spread of dengue.[7] It is estimated that there are between 50 and 100 million cases of dengue each year, of which approximately 500,000 are severe life-threatening infections.[8] There have been numerous urban outbreaks of dengue with significant health and economic impacts.[9-11] Studies in Thailand and Brazil have shown that the social and economic impact is equivalent to that of malaria in these countries.[12, 13] However globally dengue research has not received the same level of funding as other tropical infectious diseases.[8] There are currently no available drugs, no licensed vaccine and diagnosis in endemic areas is largely clinical.[14] Dengue also poses a risk to those who travel to endemic areas and is increasingly being reported in travellers returning from trips to endemic countries.[15]

Dengue viruses cause a variety of clinical syndromes ranging from asymptomatic infection to a self-limiting febrile illness to severe dengue, a life-threatening condition characterised by increased capillary permeability and shock.[16-18] There are concerns that the old WHO definitions of dengue that are still in widespread use may lead to under-diagnosis of severe dengue.[19] In clinically apparent dengue infection symptoms develop after an incubation period of 4 to 7 days with an abrupt onset of fevers often accompanied by headache with severe retro-orbital pain.[20, 21] Some patients develop severe arthralgia, explaining the historical name of break-bone fever.[22] Early in the illness the skin may appear flushed, with petechiae appearing during the 'critical phase' and a macular rash appearing in early convalescence. Even after uncomplicated dengue recovery may be complicated by fatigue and depression. Thrombocytopenia is almost universal in dengue infection and minor bleeding from skin and mucosal surfaces may be seen in uncomplicated infections - this can be severe in patients with peptic ulcer disease.[23] Biochemical hepatitis is frequently seen

in dengue infection but severe liver pathology, encephalitis and renal dysfunction are relatively rare.[21, 24] Data from Taiwan show differences in disease manifestations between adults and children observed during an outbreak in 2002.[25] Arthralgia, headache, gastrointestinal bleeding and significantly severe dengue were more common in adult patients. The reasons for these differences are not clear but could reflect differences in the host response to infection. Significant and disabling fatigue has been described after recovery from dengue.[26] It is thought that host factors may contribute to the development of this condition. Vertical transmission of dengue can occur if infection of the mother occurs within 8 days of delivery.[27] The WHO have recently published new guidelines on dengue management.[28] The new guidelines indicate that disease caused by dengue encompasses a wide clinical spectrum and that the previously used classifications for severe dengue were not always helpful. Dengue is classified in the new system as either uncomplicated or severe.[28] Severe disease is characterised by plasma leak, haemorrhage and organ impairment. Recognising warning signs for severe disease is important for successful clinical management. Warning signs include abdominal pain, evidence of fluid accumulation, hepatomegaly, and increases in haematocrit accompanied by fall in platelet count.[28] The mechanisms that lead to severe dengue are not completely understood although various hypotheses exist. Perhaps the most widely accepted hypothesis explaining the development of severe dengue is that of antibody-mediated immune enhancement where antibodies developed to a previous infection lead to enhanced viral uptake with a new infection of a different serotype.[29, 30] This and other hypotheses will be discussed in a later section of this review.

Sources of data

We searched the available literature up to April 2010 using PubMed, Web of Science and Embase using the terms: "dengue"; "dengue fever"; "dengue haemorrhagic fever"; and "dengue viruses".

Areas of agreement

As dengue has emerged as a global health threat it has received increased attention from the international public health community.[14] Essentially areas of agreement are based around addressing the lack of effective diagnostic and therapeutic options,

improving clinical care of affected patients, and developing tools to prevent dengue infection.

Areas of controversy

Is the virus itself or the host response to infection the main cause of severe disease?

The 4 serotypes of dengue can be further classified into different genotypes based on nucleotide variation.[31] Different genotypes have been associated with different levels of virulence.[32, 33] It appears that the virus may evolve during epidemics to cause more severe disease as seen in disease outbreaks in Cuba and Australia.[34, 35] It has been suggested that structural differences in strains of the virus lead to differing abilities to infect different cell types or cause severe disease.[36, 37] However given that only a small percentage of patients infected with dengue develop severe disease it is clear that host factors have a major role to play in dengue pathogenesis.[31]

Many of the clinical features of dengue infection may be due to the patients' immune response. The increased vascular permeability seen in dengue is associated with high levels of cytokines including tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin six (IL-6) and interleukin two (IL-2).[38, 39] However, it is important to note that these cytokines are elevated in many diseases yet dengue is a very specific clinical entity. Therefore the clinical features of dengue could be due to a specific constellation of these cytokines or a more complex interaction between host and virus that is yet to be characterised. Polymorphisms in TNF-α are associated with more severe disease.[40] Various other genetic factors have been associated with more severe disease: for example polymorphisms in the vitamin D receptor gene, the mannose-binding lectin gene, and various HLA class I and II alleles.[41-44] However, none of these studies have been large enough to indicate a clear association and the question of host susceptibility remains unanswered. Cytokine dysregulation and endothelial injury contribute to the pathogenesis of yellow fever, another flavivirus.[45] The pathophysiology of severe yellow fever infection remain unclear, but given similarities with severe dengue there may be overlap in the host and virus determinants of susceptibility.

Is the hypothesis of antibody-mediated enhancement sufficient to explain the development of severe disease?

Epidemiological studies have shown that severe disease is more commonly seen after secondary infections.[46] Infection with one serotype is thought to result in brief protection against all serotypes. As the levels of neutralising antibodies fall over time the non-neutralising or sub-neutralising antibodies form complexes with the new infecting virus and these complexes are taken up by Fc-receptor bearing cells. This results in increased uptake of virus, increased replication and viral load and hence a postulated increased likelihood of complications.[30] This phenomenon explains the severe disease observed in infants aged between 6 and 9 months where the low non-neutralising levels of maternally derived antibodies are thought to play a crucial role in the development of severe disease.[47, 48] These observations have led to concerns that a vaccine may potentially result in more severe disease by priming the immune system and putting individuals at risk when they are subsequently infected. These fears are ungrounded if such a vaccine produces long term neutralising protection to all 4 serotypes and those serotypes do not change over time.[3] However, severe disease can occur in primary infections and most secondary infections do not result in severe disease indicating that other factors contribute.[49,50,51] Direct evidence for antibody-dependent enhancement (ADE) contributing to the development of severe disease in humans has been difficult to demonstrate although recent work in infants and from elegant in vitro and clinical studies is starting to yield crucial insights into how ADE may contribute to pathogenesis.[51, 52]

The pathogenesis of dengue is complex and multifactorial. No hypothesis in isolation is sufficient to explain the development of severe disease. It is the interplay between virus factors and genetically determined host factors that determine the disease outcome in the individual patient.

Growing points - areas timely for developing research

Increased understanding of dengue pathogenesis

As discussed above the mechanisms that lead to severe dengue are incompletely understood. A greater and clearer understanding of disease pathogenesis including

host genetics, virus biology and immunopathology, would help the development of targeted clinical interventions.[14] Clarifying how dengue virus virulence varies between serotypes and evolves within epidemics would be helpful. Do different serotypes vary in their tissue tropism thus explaining differences in disease severity? Elucidating how the different factors within the immune system interact and contribute to disease development is essential. Too often research focuses on a single aspect of the immune response, be it antibody-mediated enhancement, T and B cell responses or the role of complement when a more integrated view, including consideration of the virus, is required.[31]

NSI is a glycoprotein secreted by dengue-infected cells. Early detection of NSI has demonstrated efficacy as a diagnostic tool.[54] Its role in dengue pathogenesis is unclear – defining this role may lend weight to its use in diagnostics. It is possible that it activates complement at endothelial surfaces thus contributing to the vascular leak that occurs in severe disease.[55] Clarifying the events that occur at the endothelial surface in dengue infection would be a major advance - the development of a suitable animal model of dengue infection would aid this progress.[56]

An increased understanding of genetic factors that contribute to disease development would help define more clearly populations at risk. Within populations an increased understanding of differing susceptibilities to disease development is necessary.[31]

Optimisation of clinical management

The commonly used WHO guidelines for the management of dengue were developed after observations in children hospitalised in Bangkok in the 1960s with dengue.[19] These guidelines have evolved over the years and have been adopted into clinical practice in endemic areas. An attempt to validate the WHO classifications for severe disease has been recently published.[57] This study showed that while the WHO classifications had good specificity for severe disease the sensitivity was lower. There is no doubt that the use of the guidelines have led to substantial mortality reductions, yet are guidelines originally developed for Thai children forty years ago still applicable today or for example in the increasing number of adult patients seen globally?[19, 58]

One of the difficulties diagnosing dengue haemorrhagic fever (DHF) using the old WHO definitions was that the criteria were too rigid.[16] Studies have shown that many cases of dengue that resulted in shock or death did not meet the WHO case definitions.[59, 60] These problems have led to the development of the new WHO guidelines that categorise dengue cases into uncomplicated or severe.[28] These guidelines emphasise the importance of recognising the warning signs of severe dengue - abdominal pain, vomiting, fluid accumulation, mucosal bleeding, lethargy or restlessness, hepatomegaly, rising haematocrit in conjunction with falling platelets.[28] These 2009 WHO guidelines include a detailed description of the clinical management of dengue including an algorithm for the management (http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf). A challenge is educating healthcare workers about the new guidelines and ensuring that they result in better clinical care.

The mortality in severe dengue remains significant particularly in infants.[50, 61] In addition higher mortality rates are seen when dengue newly emerges in regions where there is less experience in clinical management of cases, for example as seen in the epidemics in India and Brazil.[10, 62] Fluid management in dengue is difficult - the balance between too much and too little is critical and getting it wrong can be life-threatening.[63] Fluid resuscitation is not without risk and fluid overload in the recovery phase can cause acute respiratory distress syndrome (ARDS).[28] Fluid management in patients with co-morbidities or pregnancy is difficult and would benefit from further research.

It is likely that there is a significant unreported burden of mortality in remote health facilities where clinical management maybe poor. Early and careful administration of parenteral fluids is life saving in severe dengue. Ringer's lactate has demonstrated efficacy for use in dengue shock syndrome and dextran or starch have also been suggested for severe disease.[64] Successful management of severe dengue requires favourable staff to patient ratios and the ability to closely monitor patients.[28] Unfortunately the use of parenteral fluids and the intense monitoring that is required may not always be available and there is a need for research on the use of oral rehydration therapy aimed at preventing the development of shock in severe dengue.[14]

The endothelial leak seen in severe dengue is transient and if appropriate supportive therapy is given during this period mortality rates are low.[16] However in the early stages of disease clinical features are non-specific and are shared with other infectious aetiologies.[61] Making a diagnosis early in the disease would significantly aid clinical management. Additionally predicting which patients are at risk of severe disease would be a major advance. Traditionally diagnosis has relied on serology, which can take time to give useful results meaning diagnosis is often retrospective.[1] IgM against dengue can be detected after 5 days of fever - commercially available diagnostic kits based on ELISA have been assessed.[65] These have potential but have limitations, for example the persistence of IgM in some patients in endemic areas making it difficult to diagnose an acute infection. The detection of the dengue protein NSI also has proven use in diagnosis.[54] Quantifying the titre of NSI early in clinical illness may allow one to predict those at risk of developing severe disease.[66] Real-time PCR is another potential, highly sensitive diagnostic tool in dengue diagnosis.[67] However the cost of molecular tests such as PCR make it an impractical tool for use in many settings where dengue is prevalent. A rapid sensitive test that combines detection of NS1, IgG and IgM allowing diagnosis of infection throughout the illness course would be a major advance in dengue diagnostics. The development of appropriate clinical algorithms for use in resource-limited settings in parallel to the development of molecular diagnostic tools is necessary if the burden of dengue is to be adequately dealt with. A study has demonstrated the potential use of a decision tree algorithm using simple clinical and haematological parameters.[68] Testing the validity of such an algorithm in primary care settings have the potential to improve the triage and initial clinical management of patients with dengue. Detection of plasma leakage through serial ultrasound is a useful adjunctive tool in dengue diagnosis and management yet it is unlikely to be of benefit in many areas due to lack of availability.[69] Identifying additional predictors of dengue would be useful - making these into diagnostic techniques that are practical in resource-limited settings is the challenge.[70]

Vaccine development

A vaccine against dengue would be a major advance in the control of the disease. In view of the potential risks of antibody-mediated enhancement seen with heterotypic infections a successful vaccine would have to offer lasting protection against all 4 serotypes.[2] Based on the success of vaccination against other flaviviruses, for example

Japanese encephalitis and yellow fever, the development of a live attenuated vaccine had previously been the most promising prospect for vaccinologists.[71] However despite encouraging results with monovalent vaccines in clinical trials the development of a tetravalent formulation has proved problematic and development has been suspended.[3, 72-75] The leading vaccine candidate at present is a chimeric vaccine - a recombinant clone based on yellow fever vaccine strain with dengue virus membrane and envelope protein genes substituted into the construct.[76, 77] This chimeric vaccine has shown promise in Phase II clinical trials and appears safe and immunogenic. It is anticipated that Phase III trials will start in the near future.

Despite promising progress there remain unanswered questions that need to be addressed and are potential areas for research. The lack of a suitable animal model of dengue infection hinders the transfer of laboratory findings into clinical practice.[31] How do we quantify immune protection? Do tetravalent vaccine constructs hinder the development of immunity to individual serotypes? Could a dengue vaccine render populations more vulnerable to severe infection, either through antibody-mediated enhancement or via a mechanism similar to that seen with vaccines against respiratory syncytial virus?[78] How safe are dengue vaccines in different subsets of the population?[3]

In addition to the science of vaccine development and questions regarding the safety and efficacy of individual vaccine candidates there needs to be sustained co-ordination between different countries and vaccine developers if a vaccine is to be successfully developed.[79]

Anti-dengue drugs

A greater understanding of dengue virus biology has meant that targets within the lifecycle have been identified that could potentially be the site of a therapeutic agent.[80] The staggering success in developing drugs against HIV is an example of how efficiently effective antivirals can be developed given appropriate funding.

One potential mechanism of action of an anti-dengue drug is through inhibition of viral entry. The fusion of the viral membrane with the host membrane is mediated by dengue virus E protein.[81] Research has shown some promising initial results in

laboratory studies with an experimental entry inhibitor - these findings could pave the way to the development of successful therapeutic agents.[82] Other potential targets receiving research attention are the viral proteins NS3 and NS5, which play an integral role in genome replication - their protease domains could be target for protease inhibitors.[83, 84] A computer programme has been used to identify suitable molecules that can "dock" into the NS3 protease domain.[85] The challenge is to bridge the gap between findings in the laboratory and the bedside however given the progress in our understanding of dengue virus biology the future of drug development is encouraging. There are potential therapeutic developments for the treatment of other flaviviruses such as hepatitis C.[86] In view of structural similarities between different flaviviruses these developments could be used in the field of dengue treatment. The complications of dengue occur as the virus is cleared from the blood so it is possible that an anti-dengue drug will have no beneficial role. As the severe manifestations of disease are in part due to the immune response perhaps the development of an immunomodulatory agent would be the best therapeutic strategy? Animal studies have shown the protective effect of monoclonal antibodies against the NS1 protein of West Nile virus, and more recent work in mice has shown the therapeutic potential of a monoclonal antibody directed against a structural epitope in an experimental DENV-1 infection.[87, 88]

Vector control

In addition to vaccination successful vector control would be a useful adjunct to controlling dengue. Dengue is closely associated with *Aedes aegypti* - the global spread of dengue is related to changes in human behaviour, in particular the expansion of large urban centres, that support the breeding of *A. aegypti*.[6] While vector control has previously had success the persistence of pockets of mosquitoes resulted in disease re-emergence.[89] Studies have shown different efficiencies of dengue transmission between different mosquito strains and different serotypes of dengue.[90, 91] A greater understanding of the interactions between mosquitoes and the different serotypes of dengue would give us a greater understanding of transmission dynamics and would potentially lead to better prediction of epidemics and thus better control of the disease. The development of genetically modified mosquitoes that are less able to transmit dengue is a potential option for disease control. However mathematical models have indicated that this strategy may select for greater disease

virulence suggesting that measures that increase mosquito mortality may be more effective.[92] Transgenic mosquitoes are likely to be less reproductively fit - infection of transgenic mosquitoes with the symbiont Wolbachia potentially confers a reproductive advantage thus allowing the establishment of transgenics in the environment.[93] Cyclopoid copepods are invertebrate predators of mosquito larvae. Introduction of copepods into water containers, the typical breeding site of *Aedes*, can have a major impact on mosquito populations.[94] The roll out of larvicidal interventions requires active community involvement and the success of such measures would depend on imaginative public health engagement.[94]

Conclusions

Successfully tackling the threat of dengue represents a major public health challenge for the 21st century. A coordinated multidisciplinary approach is necessary. Major advances are possible provided dengue research receives the attention and funding its prevalence deserves.

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