

T Cell Immunopathogenesis of Dengue Virus Infection

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Abstract

Dengue virus infections cause significant morbidity and mortality worldwide. Although initially believed to be an infection afflicting mainly the paediatric age group, this infection has been rapidly spreading across all age groups, with the first reports of the pandemic from South-East Asia dating back to the 1950s. Dengue virus belongs to the genus *Flavivirus* and has four serotypes, each of which may cause a spectrum of illness ranging from asymptomatic to mild febrile illness to severe and often fatal haemorrhagic disease. Despite our growing understanding of various facets of the infection, its pathogenesis still remains unclear, with the possibility of several mechanisms being involved simultaneously. The virus is taken up by dendritic cells, which, after antigen processing, presents it to T cells, leading to immune activation and release of a cascade of cytokines that are believed to mediate the systemic effects of plasma leakage and circulatory insufficiency. Thrombocytopenia develops due to the presence of cross-reacting antibodies to platelets and is responsible for the bleeding diathesis. The phenomenon of 'original antigenic sin' may explain the increased severity of illness during secondary infections, due to the presence of antibody to the previously infecting serotype. This leads to immune enhancement leading to the development of dengue shock syndrome (DSS). In addition, there is evidence for increased apoptosis and endothelial cell dysfunction, which may also contribute to its pathogenesis. Dengue virus infection in infants may cause increased morbidity due to the presence of pre-existing maternal antibodies in endemic areas. Several advances have been made in the treatment of acute infections, and recent preventive efforts have been directed towards the development of safe and effective vaccines against all the four serotypes in order to provide lasting protection from this dreaded infection, which still causes high morbidity and mortality in most developing countries.

Keywords: Dengue, dengue haemorrhagic fever, dengue shock syndrome, immunopathogenesis, dengue vaccines.

Introduction

The dengue virus infection is prevalent across the tropical belt in over 100 countries, with 2.5 billion people at risk of acquiring the infection and an estimated 50 million infections and 500 000 dengue haemorrhagic fever (DHF) cases occurring annually^[1,2]. Dengue virus belongs to the genus *Flavivirus*, family *Flaviviridae* and has four serotypes, called

DENV-1, DENV-2, DENV-3 and DENV-4. It causes a spectrum of infection ranging from mild febrile illness to severe and often fatal haemorrhagic disease.

Despite our growing understanding of the various facets of dengue virus infection, the pathogenesis of dengue still remains unclear. Although several mechanisms may be involved simultaneously, their relative importance

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remains undefined. This article reviews our current knowledge of the pathogenesis of dengue virus infection and the implications of ongoing research in improving our understanding of this dreaded infection.

The publications reviewed for this article were obtained using the Medline search with terms “dengue and immunopathogenesis” and “dengue and pathogenesis”. The search was further refined by adding “children” to the above terms to specifically look for dengue infection in children <15 years of age.

Dengue fever (DF) is caused by ingestion of viraemic blood containing dengue virus by mosquitoes of *Aedes* spp. followed by passage to a second human host. An extrinsic incubation period of 8–10 days is required before the virus appears in the saliva of the transmitting female *Aedes* mosquito. After the bite, there is an incubation period of 3 to 14 days (average 4 to 7 days) followed by the onset of fever and non-specific signs and symptoms (frontal headache, retro-orbital pain, body aches, nausea and vomiting, joint pains, weakness and rash). This febrile phase is self-limiting and may last for 2 to 10 days followed by remission of fever in most cases. Laboratory abnormalities include neutropenia followed by a lymphocytosis (usually atypical lymphocytes

and thrombocytopenia. Liver enzyme levels may be mildly elevated.

Dengue haemorrhagic fever (DHF) is a severe manifestation of dengue fever and generally affects children less than 15 years of age. It is characterized by a similar initial course but there is onset of haemorrhagic manifestations and circulatory insufficiency within or just 24 hours before remission of fever. The severity is graded by the World Health Organisation (WHO) grading and grade III and IV represent dengue shock syndrome (DSS), with circulatory insufficiency in addition to bleeding manifestations. Laboratory abnormalities include leukopenia, thrombocytopenia and haemoconcentration with primary pathophysiological abnormality being an acute increase in vascular permeability leading to extravasation of fluid, haemoconcentration and hypotension. There is plasma leakage into the serous cavities also and hepatomegaly with raised liver enzymes is not uncommon. DHF and DSS require aggressive fluid therapy and supportive management for haemorrhagic manifestations but have a high mortality rate if not addressed early in their course. There are no apparent destructive vascular lesions, suggesting that a short-acting mediator may be causing transient functional vascular changes^[3]. Several risk factors for the occurrence of DHF/DSS have been identified (Table).

Table. Risk factors for DHF/DSS

- Virus strain: epidemic potential, risk of DHF is greatest for DENV-2 followed by DENV-3, DENV-4 and DENV-1
- Pre-existing anti-dengue antibody
- Age of host
- Genetics
- Secondary infection
- Hyperendemicity – two or more virus serotypes may be circulating simultaneously at high level.



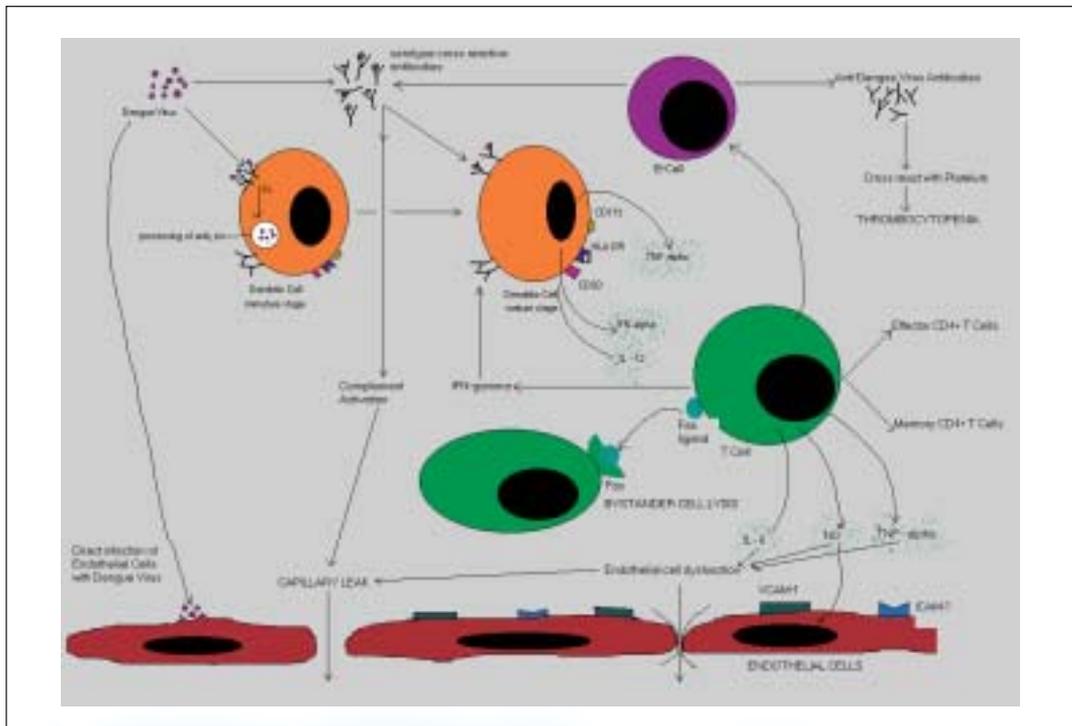
Immunopathogenesis

The first contact of the dengue virus with the immune system is when it encounters dendritic cells (DCs) in the tissues (Figure). These dedicated antigen-presenting cells (APCs) internalize the virus where it resides in cystic vacuoles, vesicles and endoplasmic reticulum. There are then two stages in DC development – the first one being immature stage, where the DCs primarily phagocytose and process the antigen. The mature stage is characterized by up-regulation of expression of CD83, other co-stimulatory and HLA DR molecules for effective antigen presentation to T cells. Once these cells undergo maturation, they release a number of cytokines, including TNF- α and IFN- α that have specific roles in pathogenesis. TNF- α and IFN- α also lead to activation of other virus infected and non-infected DCs in a paracrine manner^[4]. Simultaneously, there is

low-level release of IL-12p70, a key cytokine in the development of cell mediated immunity (CMI).

Addition of IFN- γ at this stage (in vitro) resulted in the enhancement of IL-12p70 synthesis, suggesting that IFN- γ is an important second signal for secretion of bioactive IL-12 from DCs in addition to its effect on expression of molecules involved in stimulating antigen-specific T cell response^[5]. This may be a regulatory mechanism for preventing potentially harmful Th1 type immune response early in the pathogenesis of acute dengue virus infection without the associated anamnestic burst of cytokines. During a secondary dengue infection, memory T cells would produce early IFN- γ and CD40L in the DC micro-environment leading to greater DC activation and subsequent T cell stimulation and cytokine release, especially IL-12p70. The viraemia may

Figure. **Possible immunopathogenetic mechanisms of dengue virus infection**



be cleared but the cascade of events initiated by the early, poorly controlled type 1 cytokine response contributes to the pathogenesis of DHF/DSS. Various studies have shown higher levels of TNF- α , soluble TNF- α receptor and IFN- γ levels in patients with DHF/DSS as compared to those with dengue fever.

The dendritic cells also phagocytose other virus-infected cells undergoing apoptosis. The DC maturation process can be regulated by a variety of stimuli such as cytokines, viral products and CD40 ligand. A number of organisms like measles virus, influenza virus, *P. falciparum*, *T. cruzi* etc. also modulate the DC maturation process as part of their pathogenesis to influence the immune response. These infected cells then migrate from peripheral tissues to lymph nodes and activate CD4+ and CD8+ T lymphocytes for the generation of adaptive immune response. However, most of the studies using dengue virus have used peripheral blood myeloid DCs to characterize these interactions, assuming that they are equivalent to the interstitial DCs that actually encounter the virus after the initial mosquito bite^[6]. There is some evidence of direct infection of B cells also with the virus, leading to similar pathogenetic mechanisms as in DCs, such as the synthesis of cytokines (IL-6, TNF- α) and that of cross-reacting auto antibodies against platelets and endothelial cells.

Role of T Cells

T lymphocytes have a central role in the immune response to dengue virus infection. T cell function in primary infection stage show varied patterns of T cell responses, which indicate that host response is complex and different patterns of proliferation and cytokine formation may bring about varied outcomes. Interactions between pre-existing T cell responses and other host and viral factors may also be important in determining outcome. In

a study of T cell function, subjects were Thai schoolchildren who were enrolled prospectively and their pre-illness blood samples were taken and preserved^[7]. They were then followed up for development of any episode of fever (based on their school absence) and were then evaluated and blood samples (acute and convalescent) were taken during active dengue infection (which was confirmed by virus isolation and/or RT PCR). This gave the researchers the opportunity to study the various cellular and humoral immune parameters and compare them with pre-illness sample of the same subject. If the subject's pre-illness sample had antibodies to dengue virus, then the present acute episode obviously represented secondary infection.

It also showed that a subset of patients might not develop DHF/DSS on secondary infection with dengue virus. These patients were shown to have no proliferation or cytokine response to any of the dengue antigens but had definite evidence of prior dengue virus infection in the form of detectable pre-infection neutralizing anti-dengue virus antibody titres. This was thought to be due to very low CD4 T cell response during the initial primary infection and/or T cell response having waned over time.

The serotype-specific T cells generated after primary infection recognize the more highly conserved non-structural proteins of dengue virus (especially NS3 protein). They are both of CD4 and CD8 type, with pattern and magnitude of cross-reactive response to the other serotypes being highly variable among the same subjects, even among those infected with the same virus^[8]. In addition to synthesis of cytokines to mediate their effects (discussed below), the dengue virus-specific T cells lyse other virus-infected cells by perforin-dependent mechanisms and also cause Bystander Cell Lysis of the uninfected cells. The latter effect is mediated via Fas Ligand (FasL) expressed on the surface of activated T cells that binds to



the Fas molecules on the 'innocent' bystander cells. This effect has been demonstrated *in vitro* in uninfected HepG2 cell line cultured with dengue virus-specific CD4 T cell clones.

Reversal of CD4 and CD8 ratio has been reported in a proportion of cases and their numbers are significantly higher among those with DHF/DSS. It reverts back to normal by the 15th day of illness and represents aberrant immune activation and ongoing apoptosis (discussed below). Markers of T cell activation (soluble IL-2 receptor, soluble CD4, CD8 and monokines) are found at higher levels in patients with DHF/DSS as compared to those with DF, suggesting greater T cell activation in the former^[9]. The expansion of T cell receptors using V β genes is also associated with increased severity in several diseases. In an attempt to study the biological basis of T cell activation in dengue infection, T cell V β gene usage studies were done in Thai children. It showed no difference in its expression in those with dengue fever when compared to those with DHF^[10].

Secondary Infection or Immune Enhancement Hypothesis

This is an essential component of the pathogenesis of DHF, which explains why some individuals with acute dengue viral infection present as dengue fever while others go on to develop DHF and/or DSS. Halstead proposed the mechanism of antibody-dependent enhancement whereby pre-existing non-neutralizing antibodies opsonize the virus and enhance its uptake and replication in macrophages. This leads to a higher viral load and enhanced antigen presentation resulting in extensive T cell activation. This extensive activation supposedly causes the T cells to become "stunned", whereby their IFN- γ expression remains low^[11].

Serotype cross-reactive antibodies generated from previous primary infection with a particular dengue viral serotype are not highly specific for the other serotypes involved in secondary infections. Hence they bind to the virions but do not neutralize them, and instead increase their uptake by cells which express Fc γ receptors on their surfaces - like tissue dendritic cells, monocytes and macrophages. Such antibody-coated virions are taken up more rapidly than uncoated virus particles and this leads to enhanced antigen presentation by the infected dendritic cells to the T cells, leading to the more rapid activation and proliferation of memory T cells. The cytokines produced by the activated T cells have several important effects that lead to the pathogenesis of DHF/DSS.

However, the evidence for the occurrence of antibody-dependent enhancement *in vivo* is circumstantial, as it has only been demonstrated *in vitro* in sera from infected children^[12]. Furthermore, there is no evidence from clinical studies to show that secondary infections are associated with higher titres of viremia as compared to primary infection^[13].

Role of Cytokines

Cytokines are implicated in the pathogenesis of vascular compromise and haemorrhage in dengue virus infection. The evidence for their involvement came from the observations of Innes, who suggested that the lack of structural damage, short-lived nature of the plasma leakage and remarkably rapid recovery suggest that altered permeability may be due to a soluble mediator^[14].

The release of cytokines in response to dengue viral infection includes both inflammatory as well as inhibitory cytokines and the net outcome will depend on the balance between various cytokine actions. There is an



overall increase in the levels of T cell activation markers such as soluble IL-2 receptor, soluble CD4 and CD8, IL-2 and IFN- γ as well as that of monokines like TNF α , IFN- β and GM-CSF, and these levels were consistently higher in those with DHF/DSS as against those with DF^[15]. IL-6 has dual role of being pro- and anti-inflammatory and it shows transient high elevation on day 7 or day 9–11 after onset of fever^[16]. Dengue patients with shock have significantly higher levels of IL-6 at admission as compared to normotensive patients and it is also associated with higher incidence of ascites. Similarly, high levels of IL-8 were recovered from the serum and pleural fluid of patients with DSS^[17]. Chaturvedi et al. have also postulated the presence of a unique cytokine called human cytotoxic factor (HCF), which is believed to initiate a series of events leading to a shift from Th1-type response in mild illness to Th2-type response that leads to DHF^[18].

Original Antigenic Sin

This phenomenon is applicable to secondary challenge by a pathogen where the response is dominated by the proliferation of cross-reacting memory cells which were initially induced by the primary infection, but they may be of lower affinity to the secondarily challenging pathogen. The T cell responses for secondary dengue viral infections also demonstrate this phenomenon, which may eventually delay or suppress viral elimination, leading to higher viral loads and thus increased immunopathology^[19]. The initial evidence for this phenomenon came from the observations that in secondary infections with DENV-1, many T cells showed a preference for DENV-3 tetramers and in DENV-2 infections there was better reaction with DENV-1 and DENV-3 tetramers initially. The response increased in later samples suggesting that these cells were

proliferating in response to the currently infecting virus and that it could induce the proliferation of a spectrum of clones with differing affinities and cross-reactivities. Therefore, secondary infection with a virus carrying a similar but distinct epitope stimulates the proliferation of cross-reacting, low-affinity clones and this characterizes the phenomenon of original antigenic sin.

Further insights were provided into this mechanism by the work of Screaton et al.^[19] who studied the virus-specific T cell responses in acutely infected Thai children. It was found that few dengue-responsive CD8+ T cells were found during acute infection and most of these had a low affinity for the infecting virus serotype. That is, the original exposure to the dengue virus of a different serotype caused the T cell responses to become suppressed, thus delaying viral elimination and leading to higher viral loads, with possible increased immunopathology. The authors proposed that profound T cell activation and death may contribute to systemic disturbances leading to DHF.

Apoptosis

The dengue virus infects the monocyte/macrophage cell lineage and the ability of dendritic cells (DCs) to shape the adaptive immune response to viral infection is mediated largely by their maturation and activation state, as determined by the surface expression of HLA molecules, costimulatory molecules and cytokine production^[20].

After entrance, dengue virions can be visualized in cystic vesicles, vacuoles and the endoplasmic reticulum. The dengue virus-infected DC also shows proliferation and hypertrophy of the endoplasmic reticulum as well as swollen mitochondria^[21].



Viral replication occurs within these cells and also leads to further dissemination of the virus. Once the cell is activated, it releases a number of soluble mediators including TNF- α , which alter the biological properties of endothelial cells. More specifically, it is also believed to induce apoptosis among these cells as an indirect attempt to limit viral replication. The study of human monocyte cultures infected with DENV-2 viruses showed increased production of TNF- α and increased apoptosis, but the production of nitric oxide was not increased^[22].

In addition, there is evidence to show that endothelial cells also undergo apoptosis, which causes disruption of endothelial cell barrier, leading to the syndrome of generalized vascular leakage^[23]. Antibodies against viral protein NS1 (non-structural protein) generated in mouse animal model cross-react with mouse as well as human endothelial cells. The binding of these antibodies to endothelial cells led to an increased expression of inducible NO synthase (iNOS) and subsequently increased production of nitric oxide (NO). Further evaluation of the signaling pathways showed the involvement of caspase-dependent mechanism, which involved up-regulation of pro-apoptotic factors (p53 and Bax) and down regulation of anti-apoptotic factors (bcl-2 and bcl-xl) in the endothelial cells. These changes were not observed when inhibitor of iNO (L-NAME) was added to the cells, suggesting the role of NO in inducing endothelial cell dysfunction and plasma leakage.

Thrombocytopenia in Dengue Fever

Dengue viral infection is commonly associated with thrombocytopenia, the cause of which is molecular mimicry between dengue virus proteins and endogenous self-proteins. There

is generation of antibodies against dengue virus proteins (especially NS1), which cross-react with platelet surface proteins and thus cause thrombocytopenia^[24]. These antibodies, besides causing platelet lysis via complement activation, also inhibit ADP-induced platelet aggregation^[25]. The titre of these IgM antibodies has been found to be much higher in patients with DHF/DSS than in those with dengue fever.

Endothelial Dysfunction in Dengue Fever

Endothelial cell dysfunction in dengue virus infection manifests as diffuse increase in capillary permeability, which is responsible for the microvascular leakage, haemoconcentration and circulatory insufficiency. The transient nature of plasma leakage suggests that it's probably mediated by a soluble mediator. Studies have shown that infection of endothelial cells with dengue virus induces transcriptional up-regulation and secretion of RANTES and IL-8 and extremely high levels of IL-8 have also been detected in plasma and pleural fluid samples from patients with DSS^[17].

Assessment of microvascular leakage using strain gauge plethysmography in children with DHF and DSS found that although the coefficient of microvascular permeability (K_f) was higher in children with either DHF or DSS as against healthy controls, there was no significant difference in the two groups as well, suggesting similar pathogenetic mechanism. Since each recording takes 45 minutes, the measurements in children with DSS were made after the initial fluid resuscitation. However, the fluctuations in K_f were larger in children with DSS as against those with DHF, leading to short-lived peaks of markedly increased microvascular permeability and consequent circulatory insufficiency. Irrespective of the presence of shock, all patients with dengue



virus infection demonstrate volumotion, which implies slow-wave cyclical changes in limb circumference probably due to underlying vasomotion – i.e. periodic change in arteriolar diameter. The latter could be a compensatory mechanism to improve tissue oxygenation and blood flow to prevent hypoxic damage in the face of circulatory insufficiency. The authors postulated the presence of a probable hypothetical threshold for shock, beyond which increases in microvascular permeability result in DSS^[26].

Effect of Maternal Antibodies

Infants less than one year of age who acquire maternal anti-dengue IgG antibody may be at risk of DHF/DSS even during primary infection^[27]. At the same time, the occurrence of sequential primary infection with different virus serotypes in these infants cannot be ruled out. Hence, in the presence of pre-existing maternal antibodies against dengue virus in these infants, the occurrence of even primary infection in this age group can lead to severe manifestations due to the phenomenon of immune enhancement.

The data from recent epidemics from India show variable rates of occurrence of DHF/DSS versus dengue fever in the age group of <1 year. In the Delhi epidemic of 1996, 9% of infants presented with signs of DHF, the youngest one being 3 months of age^[28], while data from the recent Chennai epidemic (September 2001–January 2002)^[29] showed higher proportion of DSS in infants as compared to that in older children (overall 20% of cases being infants). The Chennai study also showed a higher incidence of convulsions, drowsiness, orbital puffiness and rash in infants; while vomiting, restlessness and abdominal pain were predominant in older children. Whether the occurrence of convulsions in infants was due to fever or dengue infection per se cannot

be truly determined but a higher incidence of convulsions in infants with dengue has been reported by others. The infants were also found to have more pronounced thrombocytopenia but the complications due to bleeding were less pronounced than in older children^[30].

Recent Advances in Therapy

As our understanding about the pathogenesis of dengue virus infection improves, new interventions might help improve the outcome of this oft-fatal infection. The recent use of anti-TNF- α serum in the mouse model of DENV-2 infection reduced the mortality rate significantly (from 100% in untreated to 40% in treated animals, $P < 0.05$)^[31]. This intervention was based on the fact that TNF- α was significantly raised in patients with DHF/DSS as against those with dengue fever. However, it may take a while before this promising approach could be applied in humans as well. Other drugs that have been tried include ribavirin, a guanosine analogue for RNA viruses, which has been shown to inhibit dengue virus replication and IL-6 and IL-8 production in endothelial cells. Amantadine has also been tried and carboxyfullerene, a novel compound with anti-oxidant properties, has the ability to inactivate the dengue virus in a light-dependent manner.

There has been an attempt to reduce the mortality associated with DSS by using more aggressive fluid therapy than that suggested by WHO. Although no significant differences were demonstrated among the various fluid types used for the initial resuscitation, but colloids' use may be associated with most rapid normalization of haematocrit and restoration of cardiac index^[32].

An alternative protocol suggests the use of greater amounts of fluids, and, there has been interest in the use of aggressive fluid



removal strategies of the excess fluid to decrease the overall morbidity and mortality in children with DHF^[33].

In addition, there has been ongoing development of peptide inhibitors of dengue virus infectivity which hold promise for development as antiviral peptide drugs for these infections^[34]. These peptides mimic the alpha portion of the viral fusion proteins, which help to mediate cell entry of the virus and thus check the entry of the virions into cells. Another exciting group of compounds, the alpha-glucosidase inhibitors, comprising of castanospermine and deoxynojirimycin, inhibit type virus infection both in vitro and in vivo, possibly by disrupting the folding of structural proteins prM and E, a step which is crucial for viral secretion^[35]. Two sulfated polysaccharides obtained from the red seaweeds, *Gymnogongrus griffithsiae* and *Cryptonemia crenulata*, have also shown activity against dengue virus serotype 2 and the initial processes of virus adsorption and internalization are the main targets of these compounds^[36].

Vaccines

Dengue viruses are small, single-strand, positive-sense RNA Flaviviruses with similar genetic organization and replication strategies as those of yellow fever and Japanese encephalitis viruses^[37]. By replacing the pre-membrane and envelope genes of yellow fever 17D vaccine with those of Japanese encephalitis (JE) virus, unique live-attenuated chimeric vaccines have been generated for JE by Thomas Monath and colleagues^[38]. This vaccine had no unusual side effects and generated high titres of neutralizing antibodies to JE virus. A similar approach has also been tried for dengue by making chimera vaccines with all four dengue virus strains. At present, there are six vaccines in late stages of development – four of them being chimera

vaccines and the other two have been made by passaging dengue viruses in non-human tissue cultures.

The first vaccine was developed at Mahidol University in Bangkok, Thailand, and licensed by Aventis Pasteur. It produces 80–90% seroconversion rates to all four serotypes after the administration of two doses in young children^[39]. The second vaccine, produced by the Walter Reed Army Institute of Research, USA, and licensed by GlaxoSmithKline, produced similar seroconversion rates in adult volunteers. But the molecular basis of attenuation by these vaccines is not understood and it is believed that interference in replication between the serotypes and/or interference in immune stimulation may lead to imbalanced immune responses resulting in incomplete protection and enhanced disease severity. In addition, reversion to virulence through mutation or recombination between the vaccine components or with wild virus, are causes for concern.

The most recent evaluation of DENV-2 inactivated, recombinant subunit and live-attenuated vaccine candidates in the rhesus macaque model^[40] has shown encouraging results. While this data is from animal studies only, it represents an important step towards demonstrating the safety, efficacy and immunogenicity of these potential vaccine candidates.

Considering the disease burden and significant dengue-associated morbidity and mortality, a review of the cost-effectiveness of dengue vaccines found their implementation to be cost-effective. A meeting of the Pharmaco-economic Working Group^[41] concluded that it would cost \$0.50 per dose in the public sector and \$10 per dose in the private sector, the overall average cost for vaccinating one child being \$7.58. The gross cost of the vaccination programme per 1000



population in South-East Asia would be \$154 and the overall cost per disability-adjusted-life-year (DALY) saved would be \$50. In view of the overall savings in health care costs due to reduction in cases of DHF, the net cost per capita would be \$17. This data is from 10 South-East Asian countries (Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand and Viet Nam) and does not include India, so there is a need to evaluate the disease profile in our country and formulate similar cost-effective measures to assess the feasibility of vaccine implementation.

Paediatric Dengue Vaccine Initiative (PDVI) is a forum for coordination of global efforts in dengue vaccine development under the aegis of International Vaccine Institute, Seoul. It was launched in February 2002 and has been active in collecting information on the disease burden, drafting vaccine research and development proposals and influencing policy-makers'

decisions in affected countries of South-East Asia^[42].

Conclusion

The dengue pandemic started some time during World War II and has spread progressively to involve almost all tropical countries. It has caused about 5 million hospitalizations in children and about 70 000 deaths from DHF/DSS. Widespread failure of mosquito control programmes has led to expanded efforts in the development of the tetravalent dengue vaccine. It is hoped that in the coming 10 years this public health menace could be checked by the use of safe and effective vaccines. As we gain more insight into the pathogenetic mechanisms of dengue virus infections, we can hope to improve our efforts towards providing better case management to reduce its overall morbidity and mortality and assist in the development of safe and effective vaccines against this dreaded disease.

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