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REVIEW ARTICLE

Dengue Virus Infection – Current Overview and Vaccine Update

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ABSTRACT

Dengue fever and dengue haemorrhagic fever are important arthropod-borne viral diseases. Each year, there are fifty million dengue infections and 500,000 individuals are hospitalized with dengue haemorrhagic fever, mainly in Southeast Asia, and the Pacific. India experiences epidemics of dengue fever every year and the dengue season coincides with the monsoon rains. All the four types of dengue viruses have been reported from different states. Hence it is important to understand the pathogenesis and transmission of dengue virus for proper management. This review article focuses on the current overview of mode of transmission and pathogenesis of dengue virus with a special update on available dengue vaccines.

KEY WORDS : Dengue Virus, Dengue Haemorrhagic Fever, Dengue Vaccines

Introduction

Dengue virus (DENV) belongs to the family Flaviviridae, genus Flavivirus, and is transmitted to humans by Aedes mosquitoes, mainly Aedesaegypti. Based on neutralization assay, four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) can be distinguished. DENV infection is endemic in tropical and subtropical areas, with an estimated 50 million infections occurring each year and more than 2.5 billion people being at risk of this infection [1]. Infection with any of the DENV serotypes may be asymptomatic in the majority of cases or may result in a wide spectrum of clinical symptoms,[2] ranging from a mild flu-like syndrome known as dengue fever [DF] to the most severe forms of the disease dengue hemorrhagic fever [DHF]. The latter may progress to hypovolemic shock dengue shock syndrome [DSS]. In Asia the risk of developing severe disease is greater in DENV-infected children (less than 15 years) than in adults[3-7].

India experiences epidemics of dengue fever every year and the dengue season coincides with the monsoon rains. All the four types of dengue viruses have been reported from different states. In New Delhi, 2,557 dengue cases and three deaths have been recorded

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so far this year. But the areas worst hit this year are in the southern states, according to the Health Ministry, with Kerala registering 7,000 cases and 23 deaths. In Andhra Pradesh over 5,680 were infected, 12 of them died, Orissa had 5,012 cases and five deaths and in Tamil Nadu had 4,294 dengue patients with no deaths. Elsewhere, the western states of Gujarat and Maharashtra also recorded over 2,600 cases each and a handful of fatalities (Ministry of health and family welfare)[8,9]. The aim of this review article is to provide an update on the current understanding of the dengue infection and the progress that has been made in vaccine development.

Virus transmission cycle

The enzootic transmission cycle of dengue viruses involves Aedes mosquitoes and lower primates in the rain forests of Asia and Africa. If DENV virus is spread to humans then the virus quickly infect the majority of susceptible individuals. Aedes(Stegomyia) species may act as a vector such as A. aegypti, A. albopictus, A. polynesiensis and other members of the A. scutellaris group. The most important transmission cycle from a public health standpoint is the urban endemic/epidemic cycle in large urban centers of the tropics. The viruses are maintained in an A. aegypti – human- A. aegypti cycle with periodic epidemics. Often, multiple virus serotypes co-circulate in the same geographical area (hyperendemicity)[10].

Virus attachment, penetration and uncoating

Dengue viruses attach to susceptible cells by either of two mechanisms. In one case, dengue viruses complexed to non-neutralizing, but antivirion, immunoglobulin G (IgG) antibodies may attach to macrophages or monocytes via Fc receptors found at cell surfaces. Alternatively, dengue viruses may attach to cells, including monocytes, via a trypsin-sensitive virus receptor. The composition and structure of this host cell receptor, which has been hypothesized to bind to distinct regions of the E glycoprotein, are unknown.The attached infectious virus may penetrate host cells. The virion envelope fusing with the plasma membrane with immediate deposition of the nucleocapsid into the cytoplasm, or the plasma membrane may invaginate, forming an endocytotic vesicle (endosome) around the still enveloped virus.

Viral RNA Replication

RNA replication can be detected as early as 3 h post infection and appears to occur in the peri-nuclear region of the infected cell in association with smooth membranes. Three forms of RNA can be extracted from dengue virus-infected cells and isolated by sedimentation through sucrose gradients: RNase-resistant 20S to 22S RNA called replicative form; partially RNase resistant, heterodisperse, 20S to 28S RNA called replicative intermediate; and RNase-sensitive 42S RNA. Both replicative form and replicative intermediate RNA may serve as precursors to 42S viral RNA .

Synthesis of Viral Proteins and assembly

Viral structural and nonstructural proteins are derived from a large precursor polyprotein, encoded by a long, open reading frame. Translation begins at the first AUG codon of the RNA genome, and individual viral proteins are formed by co translational proteolytic processing of the precursor peptide. The C protein is the first protein synthesized.Assembly of dengue viruses has the following phases: (i) assembly of nucleocapsids from C protein and RNA; (ii) "budding" of nucleocapsids

through membrane containing integral E and prM proteins to acquire an envelope; (iii) exit from the cell, either as a result of the budding process or, afterwards, in exocytic vesicles, and (iv) cleavage of the prM protein, resulting in a reorganization of the virion surface.

Dengue virus pathogenesis

The mosquito vectors, principally Aedesaegypti, become infected when they feed on humans during the usual five-day period of viraemia. The virus passes from the mosquito intestinal tract to the salivary glands after an extrinsic incubation period, a process that takes approximately 10 days and is most rapid at high ambient temperatures. Mosquito bites after the extrinsic incubation period result in infection. In the skin, dengue viruses infect dendritic cells through the non-specific receptor dendritic cell specific ICAM3-grabbing non-integrin (DC-SIGN)[11]. Infected dendritic cells migrate to local or regional lymph nodes where they present viral antigens to T cells, initiating the cellular and humoral immune responses. There is also evidence of abundant replication of DENV in liver parenchymal cells and in macrophages in lymph nodes, liverand spleen, as well as in peripheral blood monocytes[12]. Both invitro and invivo, macrophages and monocytes exhibit antibody dependent enhancement (ADE) of infection. ADE occurs when monocytes are infected through their Fc receptors by immune complexes that form between DENVs and non-neutralizing antibodies. These non neutralizing antibodies result from previous heterotypic dengue infections or from low concentrations of dengue antibodies of maternal origin in infant sera. The co circulation of four DENV serotypes in a given population might augment the ADE phenomenon[13]

Role of soluble factors in pathogenesis of Dengue

It is strongly believed that a high viral load and activation of high numbers of non-protective T cells result in a "storm" of inflammatory cytokines and other mediators, leading to the increased plasma leakage characteristic of DHF/DSS. One of the most daunting challenges in DENV research is the identification of soluble factors that can mediate, the functional changes induced in EC that are associated with the increased plasma leakage. Several studies have shown that concentrations of multiple cytokines and other mediators, as well as soluble receptors, are significantly increased during severe dengue infections. Higher plasma levels of IL-1α, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-18, TGF-1, have been reported in DHF and DSS[14]. The different soluble factors are C3a, C5a, C4b, IL-1, IL-6, IL-8, IL-10, TNF-α, TGF-β, VEGF are associated with the pathogenesis of dengue.

Factors Responsible for the Increased Incidence of Dengue fever

The factors responsible for the dramatic resurgence and emergence of epidemic dengue and DHF, respectively, as a global public health problem in the past 17 years are complex[15,16]. First is the unprecedented global population growth and the associated unplanned urbanization, especially in tropical developing countries. Second is the substandard housing and deterioration in sewer and waste management which created ideal conditions for increased transmission of mosquito-borne diseases. Third is the lack of effective mosquito control in areas where dengue is endemic[17,18].Population densities of A. aegypti have increased, in urban areas of the tropics, because of increased numbers of mosquito larval habitats in the domestic environment such as non- biodegradable plastics and used automobile tires.

Dengue in Indian scenario

DF has been known to be endemic in India for more than 20 years as a benign and self-limited disease; however, during recent years, the severe form as DHF is manifesting more frequently. The first serotype isolated in India was Den-1 in the year 1945 from Kolkata. However, large and severe outbreaks in India have been mostly caused by dengue virus type-2[19]. Delhi has had outbreaks of dengue caused by various dengue virus types in 1967, 1970, 1982, 1988 and 1996[20]. Dengue virus types 1, 2 and 3 have all been isolated during previous dengue outbreaks in Delhi, but a particular type has always predominated. One of the largest outbreaks of DHF/DSS in North India occurred in Delhi and adjoining areas in the year 1996 and the predominant circulating serotype was found to be Den-2 virus[21,22]. These findings indicate replacement of the earlier circulating serotype Den-2 with Den-3. There was co-circulation of all four serotypes in the year 2007, indicating continuous co-circulation of all four serotypes, leading to hyper endemicity of dengue. In India Den-3 was the major circulating serotype in children, while Den-2 was the predominant one in adults.

Laboratory diagnosis of Dengue fever

IgM antibodies against dengue virus (after 6 days) can be detected in most cases, followed by IgG antibodies by day 14. In a secondary infection with other serotype these ab appear much early. During the viraemic period, the reverse transcriptase-polymerase chain reaction (RT-PCR) and tests to detect nonstructural 1 (NS1) protein should be used [23- 25]. The detection of dengue viral RNA by RT-PCR is performed in many research and some reference laboratories. Variety of serological tests have been used to measure anti-dengue antibodies; each measuring different antibody activities. The haemagglutination inhibition assay (HIA) has been considered the gold standard for detecting dengue-specific antibodies and it can be used to identify past infection and to differentiate between primary and secondary dengue infections, but it cannot reliably differentiate between dengue and other flaviviruses(such as Japanese encephalitis and yellow fever viruses), both of which occur in dengue endemic countries, nor can it measure dengue serotype-specific immunity. Currently, the standard test used by most diagnostic laboratories is the detection of dengue-specific IgM antibodies by IgM-capture ELISA. The IgM-capture ELISA can be performed by ELISA kits, using lateral flow (LF) or immunochromatographic strip test devices for rapid point-of-care (POC) testing. The timing of the collection of the acute sample can impact on the results of the test. False negative results are common if the sample is collected too early in the infection, and because the IgM response to a secondary dengue virus infection can be low. False positive results also occur because the cross-reactivity between all flavivirus antibodies complicates distinguishing an acute dengue infection from other flavivirus infections or from other nonspecific antibodies[26].The plaque reduction neutralization test (PRNT) is used to measure virus-specific and serotype-specific neutralizing antibody against dengue, both of which are correlated with protection against dengue virus infection[27].

Challenges of Dengue vaccine development

The ideal dengue vaccine should provide long-term homotypic and heterotypic protection. Therefore, there are several factors which require consideration. First, the vaccine must be protective against each of the four DENV serotypes to reduce the risk of ADE. Second, the immunization should be safe and not cause unacceptable side-effects caused

by cross-reactive Abs or cross reactive T cells. Third, the cost should be affordable to the individuals who most need the vaccines [28,29]. There are still several obstacles for the development of dengue vaccines. One is that the complicated pathogenesis is not fully understood. Another hindrance is the lack of suitable animal models. DENV can infect nonhuman primates but does not replicate well or cause marked disease. For reasons of cost and convenience, mouse models have been used to test vaccine candidates prior to testing in nonhuman primates. In general, immune-competent mice are the more suitable models to test the immunogenicity of a vaccine. However, DENV replicates poorly in these mice. Recent progress has been made in modeling dengue in mice, using transgenic, knockout and humanized approaches[29].

Although no licensed dengue vaccine is yet available, several vaccine candidates are under development. These include live attenuated virus vaccines, live chimeric virus vaccines, inactivated virus vaccines, and live recombinant, DNA and subunit vaccines[37,38]. Live viral vaccines have advanced to clinical trials, but have shown problems, such as unequal immunogenicity of the four serotypes and viral interference among the four serotypes in tetravalent formulations. Non-viral vaccines have also been proposed and developed for safety reasons. This includes subunit vaccines that mostly focused on the E protein or its derivatives. The difficulty of eliciting balanced levels of neutralizing Abs to each of the four serotypes remains a major concern [39].

Conclusion

Many of the Dengue affected countries are poor and developing. Realistic approaches for their infrastructure development are required to be urgently addressed. Detailed serological and virological studies of dengue outbreaks in endemic areas are required to

Types of current status of Dengue vaccines, its applications and drawbacks

Dengue vaccine	Characteristics	Drawbacks
Live attenuated vaccine	• Serial passage of dengue virus in primary dog kidney- tetravalent vaccine • Serial passage of dengue virus in tissue culture- Wlter Reed Army Research Institute • Site directed mutagenesis of DENV, done byΔ 30 nucleotide deletion -DENV4Δ	• Produced adverse reactions [30] • Unbalanced immunogenicity and reacto genecity [31] • Not successful against DENV2 and DENV3 [32]
Live chimeric virus vaccines Sanofi Pasteur's ChimeriVax Dengue tetravalent vaccine (CVD 1to 4) [33]	• Backbone of YFV17D (Licensed) expressing the prM and E genes of all 4 DENV serotypes	• Successful Phase I trial but only 30% Success rate in Phase II trial. • Ineffective against DENV2 • Requires modification in dengue endemic countries
Inactivated virus vaccines	• Purified, inactivated DENV2	• Lack of the immunity to NS proteins • Requirement of adjuvant for enhancing [34,35]
Live recombinant DNA and subunit vaccines [36]	• DENV E protein is used as the major immunogen. • Truncated E proteins (DEN-8E) produced for all serotypes • Developed with aluminum hydroxide (adjuvant) as tetravalent vaccine formulations	• Cross reactivity with host proteins • Needs further investigations

pinpoint the nature of the outbreaks to help to develop effective control measures. Well-targeted population-based epidemiological studies with clear operational objectives are urgently required to control high morbidity and mortality due to dengue. Greater attention is to be given for development of safe, effective affordable vaccine for DENV.

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