Dengue Vaccine: A Road to Dengue Prevention

Alfia Alim[1], Asim Sarfraz[2]

ABSTRACT

Every year, thousands of deaths occur due to dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). The control of the mosquito vector, *Aedes aegypti*, is currently the only method available to prevent dengue infections. Since vector control strategies alone have not been able to satisfactorily achieve reduction in viral transmission, the implementation of a safe, efficacious and cost-effective dengue vaccine is of top priority in public health. However, dengue vaccine development has been complicated due to the unique and complex immunopathology of dengue. Dengue vaccines have also been challenged by critical issues like absence of suitable markers of protective immunity and lack of animal models for the disease. Dengue vaccines must provide solid and long lasting protection against all four dengue viruses in dengue-endemic countries, otherwise there is the risk of sensitising recipients to severe disease. Many candidate dengue vaccines are moving towards clinical trials in human beings. Despite various limitations, collaborative effects of World Health Organization with vaccine manufacturers and policy makers, to facilitate vaccine development, can make a safe and efficacious dengue vaccine a reality in near future.

Keywords: Challenges, Live-Attenuated, Types, Status, Where Next

INTRODUCTION

Dengue is a mosquito-borne virus which is most extensively spread worldwide. In the last 60 years the cases of clinical dengue reported to WHO has increased 30-fold, with a much increased geographic range and expansion towards rural settings.[1] Dengue cases reported yearly to WHO has increased from 0.4 to 1.3 million in the decade 1996–2005, reaching 2.2 million in 2010 and 3.2 million in 2015.[2,3] There is substantial under-reporting of dengue within health systems and to WHO.4 Based on mathematical modelling, the global annual incidence has been estimated at about 50 million – 100 million symptomatic cases in recent years, predominantly in Asia, followed by Latin America and Africa. In 2013 dengue was estimated to be responsible for approximately 3.2 million severe cases and 9000 deaths, the majority occurring in lower middle income countries, and for 1.1 million disability adjusted life years (DALYs) globally.[4] Since World War II, the four dengue viruses have progressively spread geographically to virtually all tropical countries to create a global pandemic resulting in several hundred thousand hospitalisations every year due to dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). Since the description of DHF/DSS in the mid-1950s, over 5 million children have been hospitalised and 70,000 have died. Hence, vaccines against dengue are needed urgently.[5] Also, with vector control as a single strategy, it has been difficult to demonstrate its effectiveness in reducing the human dengue burden. The world requires a safe and efficacious dengue vaccine.[6]

DENGUE IMMUNITY AND CHALLENGES TO VACCINE DEVELOPMENT

The disease dengue is caused by four dengue viruses (DENV), DENV-1 to DENV-4. Due to their serological and genetic relatedness they are considered four serotypes of DENV.

An ideal dengue vaccine should produce life-long protective immune response by inducing neutralising antibodies that are effective against all the four serotypes of dengue virus (DENV 1 to 4) to the same extent. However, this issue is difficult to deal with due to number of reasons.

Firstly, the phenomenon of antibody-dependent enhancement (ADE) or immune enhancement, observed in dengue virus infection due to heterologous non-neutralising antibodies, carries a potential risk of precipitating serious manifestations like DHF/DSS during secondary infection by a different serotype.[7,8] So what is ADE ? Infection with dengue virus induces the production of both neutralizing and non-neutralizing antibodies.

The neutralizing antibodies are protective in nature. Such antibodies are produced against the infecting serotype (which last lifelong) as well as against other serotypes (which last for some time). Hence protection to infective serotypes stays lifelong but cross protection to other serotypes diminishes over few months. The non-neutralizing antibodies last lifelong and are heterotypic in nature, i.e they are produced against other serotype but not against the infecting serotype. Such antibodies produced following the first serotype infection, can bind to second serotype; but instead of neutralizing the second serotype, it protects it from host immune system by inhibiting the bystander B cell activation against the second serotype. The above phenomena is known as ADE which explains the reason behind an increase in viral replication and a high level of viremia of the heterotypic virus, which is strongly associated with severe disease (DHF/DSS). [9,10]

In areas where dengue is endemic, the heterotypic antibodies which is already circulating in vaccinees in sub-neutralising

[1]Consultant, Department of Microbiology, Mahavir Cancer Institute, [2]Assistant Professor, Department of Microbiology, AIIMS Patna, India

Corresponding author: Alfia Alim, Patther ki Masjid, new Khajoorbanna, Patna-800006, India

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concentrations can increase the level of replication of the vaccine virus strains. Hence, a hypothetical risk of ADE is associated with live attenuated dengue vaccines during the brief period of viremia post-immunisation. However, the risk of severe disease due to high levels of multiplication of vaccine virus, is very low due to the attenuated phenotype of the vaccine strains.[11]

Secondly, is the phenomenon of "viral interference" which is a major safety issue observed due to ADE in vaccination with multivalent live attenuated DENV vaccines. Interference has been attributed to increased replication of one serotype of live attenuated DENV strain in the multivalent formulation that leads to unequal levels of neutralising antibodies against the 4DENV serotypes. It can be prevented by an empirical adjustment of the doses of each of the four DENV serotypes which is still a challenge in development of a tetravalent live attenuated DENV vaccine.[12,13]

Thirdly, lack of an appropriate animal model to illuminate the pathogenesis, immune response and clinical course of dengue infection in humans is one of the major challenges that have hindered the development of a safe and effective dengue vaccine.

Methods like inoculation of mouse-adapted DENV strain into suckling mice via intra-cerebral route is leading to paralysis and death of the animal.[14,15]

Fourthly, Non-human primates (NHPs), who show dengue viremia kinetics closely similar to that in humans, have been widely used for pre-clinical evaluation of dengue vaccines. However, clinical features of dengue ranging from uncomplicated fever to DHF and DSS are not manifested in NHPs. Thus the phenomena of ADE during secondary infection after vaccination cannot be evaluated using NHP models.[16]

Fifthly, neutralising antibody response to a specific DENV serotype is traditionally detected by the plaque reduction neutralisation test (PRNT). A cut-off of PRNT titre of ≥10 is taken as the marker for the presence of neutralising antibodies to a given serotype.[17]

However, the PRNT assays used for evaluation of dengue vaccines in clinical trials, were unable to distinguish homotypic from heterotypic immunity. Also, one of the drawbacks of PRNT is that the assay is performed on cell lines like Vero or LLC-MK2 which lack surface expressed Fc γ receptors. Fc γ receptors present in cells of monocyte and macrophage lineages, play an important role in DENV entry and replication into these cells. Hence immunodiagnostic assays performed on such cell lines lacking Fc γ receptors may not be true predictors of protective immune response. An additional problem with PRNT is the occurrence of inter-laboratory variations, which may account for erroneous results in vaccine efficacy trials.[18]

TYPES OF DENGUE VACCINE

Despite various challenges present for development of an ideal dengue vaccine, development of dengue vaccine candidates have progressed over the last decade through various approaches:

A classification of the current approaches for dengue vaccine development is:

Dengue vaccine candidate	Phase trial
Live attenuated dengue vaccine	Phase 3
Inactivated dengue vaccine	Phase 1
Dengue DNA vaccine	Phase 1
Dengue subunit vaccine	Phase 1
Virus vectored vaccines	Preclinical
Virus like particles (VLPs)	Preclinical

In the dengue vaccine clinical pipeline only live attenuated dengue vaccine (developed by Sanofi Pasteur) has been registered till now and the rest five candidates are in clinical development and not yet licensed.

Live attenuated dengue vaccine

CYD-TDV (Dengvaxia®) is a prophylactic, tetravalent, live attenuated viral vaccine developed by Sanofi Pasteur. The active substances present in the CYD dengue vaccine are 4 live attenuated viral recombinants which include serotypes 1, 2, 3, and 4. Each monovalent CYD recombinant is obtained separately by substituting the genes encoding the pre-membrane (prM) and envelope (E) proteins of the structural proteins in the viral genome of attenuated yellow fever (YF) 17D, with the corresponding genes of the 4 wild type dengue serotypes 1, 2, 3 and 4.

Indication - The indication of Dengavaxia is for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3, and 4 in individuals 9 through 45 or 60 years of age (depending on the licenses in Philippines, Mexico, Brazil, Paraguay and El Salvador) living in dengue endemic areas.

Dosage and route of administration - The vaccination schedule consists of 3 injections of 0.5 mL administered at 6-month intervals by the sub-cutaneous route. A time window of +/- 20 days was specified as acceptable for doses 2 and 3.

CYD-TDV is presented in a single-dose vial or in a 5-dose multi-dose vial. It is a sterile, freeze-dried product to be reconstituted before injection with either a sterile solution of 0.4% sodium chloride for the single-dose presentation or a sterile solution of 0.9% sodium chloride for the 5-dose presentation. After reconstitution, one dose (0.5 mL) is to be administered by the subcutaneous (SC) route.

Completion of the three-dose scheduled is recommended to assure the protection demonstrated in the 5-year period of trial follow up so far.

Contraindication

Individuals with congenital or acquired immune deficiency that disrupts cell-mediated immunity 2) Those with a history of severe allergic reaction to any component of the dengue vaccine 3) Pregnant or breastfeeding females 4) Individuals with symptomatic or asymptomatic HIV (Human Immunodeficiency Virus) infection when accompanied by evidence of impaired immune function.

NOTE: Administration of vaccine should be postponed in

people suffering from moderate to severe febrile or acute disease.

Advantages

These recombinant vaccines induce no or low viraemia in human recipients, which is considered below the threshold for transmission to mosquitoes. No oral infection of mosquitoes was induced, and no transmission was observed when infected directly.

Despite being a RNA virus, the YF17D vaccine genome was found to be very stable. Natural recombination (intragenetic or intergenetic) is considered unlikely, and artificial recombinants produced in the laboratory were still attenuated.

Disadvantages

While at present there is no data indicating an increased risk of hospitalization due to dengue vaccine in recipients of age group 9-45 years, there is a theoretical possibility that vaccination may be ineffective or may even increase risk of hospitalization in those who are seronegative at the time of first vaccination.

Special population

Pregnant women: Dengavaxia is contraindicated in pregnant and lactating women as insufficient data have so far been gathered on its use in pregnancy. However, based on limited data available, if a woman becomes pregnant before the administration of all three doses, the remaining doses should be administered after lactation.

Immunocompromised: the live attenuated vaccine is contraindicated in immune-compromised individuals. However in HIV-infected individuals further studies need to be done.

Travellers: the vaccine has not formally been licensed for use in travellers. In travellers previously infected with dengue, vaccination for travel may be beneficial at high transmission settings. In travellers not previously infected with dengue, vaccination may be substantially less beneficial (and there is a theoretical risk that it may be harmful), in comparison to seronegative individuals living in endemic settings. Co-administration with other travel vaccines till date, is not recommended.

Health care workers: There are no specific recommendations for vaccination to health care workers.

Proposed Recommendations

Countries should consider introduction of this vaccine in geographical areas with high dengue transmission, i.e. to individuals with seroprevalence of 70% or greater but not below 50%, in the age group targeted for vaccination.[19]

Vaccine safety

Both local and systemic side-effects were seen following vaccination with live attenuated vaccines. Solicited systemic reactions occurred in 66.5% of vaccine recipients compared to 59% of placebo recipients. The most common solicited systemic reactions were headache (>50%), followed by malaise (>40%) and myalgia (>40%).[20]

Co-administration

Co-administration studies previously conducted in children outside the indicated age range, in which CYD-TDV was co-administered with YF vaccine, DTaP-IPV/ Hib, and MMR, did not identify any safety concerns (data were comparable when vaccines were co-administered or given alone), and that the immunogenicity profiles were satisfactory both for CYD-TDV and for co-administered vaccines.[20]

Inactivated dengue vaccine

Cell culture-based inactivated whole dengue virus vaccine comprises of Vero cell grown, formalin inactivated DENV. Three doses of inactivated vaccine protected the animals against challenge with a homologous wild DENV. Although inactivated vaccines are free from disadvantages like viral interference and reversion to a pathogenic phenotype, the role of these vaccines as the sole immunisation strategy is questionable because of conformational changes in viral proteins by formalin inactivation and lack of multiplication of inactivated viruses.[21]

Dengue DNA vaccine

DNA vaccines consist of antigen encoding genes cloned into a plasmid vector, which on inoculation is taken up antigen presenting cells (APCs). This leads intracellular generation of target antigens followed by their association with major histocompatibility complex (MHC) class I and induction of protective cytotoxic immune response. DNA vaccines expressing pre-membrane (prM) and full-length E proteins were shown to be immunogenic in NHPs who were partially protected against wild DENV challenge.

Since this vaccine did not show adequate protective immune response it is being redesigned using a novel adjuvant.[22]

Dengue subunit vaccine

Various viruses such as replication-defective adenovirus vectors, Venezuelan equine encephalitis (VEE) virus vector and attenuated measles virus, have been used as vectors for insertion of DENV genes and expression of DENV antigens, capable of eliciting neutralising antibody response. Bivalent constructs expressing prM and E proteins from two serotypes each (DENV-1 and -3 in one and DENV-2 and -4 in another) have been prepared by insertion into an adenovirus vector (cAdVax). Inoculation of NHPs at separate sites lead to production of neutralising antibodies to respective DENV serotypes.

However, prior immunity due to past adenovirus infection or exposure to the measles vaccine virus can limit replication and immune responses.[23]

Virus like particles (VLPs)

Indian scientists have developed a DENV-2 E protein based non-infectious virus-like particle (VLP) using the yeast *Pitchia pastoris*, as the expression system at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi. The DENV-2 E VLP induced high titres of neutralising antibodies in murine models. Once its efficacy and safety are established, the inexpensive VLP-based formulation can be used in resource-limited dengue

endemic countries like India.[24]

CONCLUSION

Efforts for dengue vaccine development have faced multiple challenges, including the need to induce a balanced and lasting immunity against four DENV serotypes, lack of suitable animal model and potential immune enhancement of disease. However, considerable progress has been made in recent years, which has resulted in an advanced dengue vaccine pipeline, with a lead candidate entering phase 3 clinical trials and several other candidates in earlier stages of clinical evaluation. However, some uncertainties around the utilization of LAVs remain, including potential imbalances in immunity due to immune interference.

Where next? To ensure the introduction of safe and effective dengue vaccines, ways must be found in order to accelerate phase 3 trials of tetravalent dengue vaccines in the desired population. Testing in man only, can answer questions of safety and efficacy. Efforts are needed to understand protective mechanisms in dengue infections, especially those mediated by antibodies. The biggest restriction to the solution of these problems is inadequate funding, which is now being addressed by a new international consortium.

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