Dengue Vaccine: The Current Status

Maj MS Mustafa*, Lt Col VK Agrawal†

Abstract

Dengue fever is a re-emerging public health problem with two-fifths of the world population being at risk of infection. Since there are no antiviral drugs available against the dengue virus, and vector control programmes have been largely unsuccessful in preventing outbreaks, vaccination seems to be the most viable option for preventing infection. An ideal dengue vaccine should provide long lasting immunity against all four serotypes of the virus. The envelope protein of the virus plays a key role in vaccine development. The present day candidate vaccines includes a live attenuated tetravalent vaccine, intertypic chimaeric vaccines based on live attenuated dengue virus vectors, chimaeric vaccines based on the live attenuated Yellow Fever 17D vector and recombinant vaccines which include vaccines based on flavivirus and non-flavivirus vectors. Tetravalent live attenuated vaccines, intertypic chimaeric vaccines and chimaeric vaccines are being tested in human trials. Recombinant DNA vaccines based on flavivirus and non-flavivirus vectors are being tested in animal trials. Recent studies have shown that the tetravalent formulations may elicit an unbalanced immune response. Research is continuing to find means of obtaining a balanced response to all antigens in the tetravalent formulations.

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Introduction

Dengue fever has re-emerged as a major public health problem in South East Asia and Latin America. It is estimated that there are more than 100 million cases of dengue fever, 5,00,000 cases of dengue haemorrhagic fever (DHF) and 25,000 deaths attributable to dengue annually, with 2.5 billion people in over 100 countries at risk of infection [1]. In India, there has been a dramatic rise in the incidence of dengue fever cases. In the year 2006, 10,034 cases were reported in the country, of which 175 were fatal.

Currently, there is no sustainable preventive approach against dengue. Community-based mosquito control projects have succeeded only on a small scale. National vector control programmes have been largely unsuccessful or of only short-term local benefits. Thus, vaccine development continues to be potentially the most effective control strategy.

Vaccine Development : Problem Areas

Even after more than sixty years of research, a licensed vaccine against dengue is still elusive and even today there are only "candidate" dengue vaccines.

There are four serologically distinct viruses (DEN1, DEN2, DEN3 and DEN4) and long-term immunity is specific for the infecting serotype. Sequential infections with different serotypes may lead to catastrophic forms

of the disease. Hence, protection against only one or two dengue viruses may in fact increase the risk of potentially fatal DHF and dengue shock syndrome through antibody dependent enhancement of infection [2]. In addition, there is an extensive evidence of intraserotypic recombination occurring in dengue viruses, which may give rise to a novel virus [3]. The lack of a suitable animal model is a major obstacle to the study of dengue viral pathogenesis.

Dengue Virology

The dengue virus is composed of viral genome and three structural proteins. The viral genome is positive-sense RNA, i.e., if naked viral RNA enters a cell, viral proteins and eventually viral particles would be produced. This is in contrast to negative-sense RNA viruses which require the presence of a polymerase for viral proteins to be made. The three dengue viral structural proteins are the capsid protein, the pre-membrane (prM) protein and the envelope (E) protein [4]. The E protein is particularly important for vaccine development as it mediates virus entry by interacting with host cell surface receptors and is also the primary target against which neutralising antibodies are directed by the body. Expression of dengue virus proteins has been achieved by making complementary DNA (cDNA) and determining the genetic code of the virus. Through in

*DADH, HQ 6 Mtn Div, C/o 56 APO, Pin - 908406. †Associate Professor, Department of Community Medicine, Armed Forces Medical College, Pune-411040.

vitro manipulation, the entire cDNA sequence of the virus has been cloned using *E coli* host/vector system [5]. Investigators have succeeded in making infectious cDNA-viral RNA, which can be used to infect cells and produce novel dengue viruses, which forms the basis of dengue vaccine development.

Research into dengue vaccines focusses on the use of tetravalent live attenuated vaccines, intertypic chimaeric vaccines, chimaeric vaccines and recombinant DNA vaccines based on flavivirus and non-flavivirus vectors (Table 1).

Tetravalent Live Attenuated Vaccine

The most advanced live attenuated tetravalent vaccine has been developed at Mahidol University, Thailand. Attenuated viruses of all four serotypes were developed by serial passage of wild-type viruses in primary dog kidney (PDK) cells. The vaccine has successfully completed Phase 2 clinical trials, which have shown that it is safe and immunogenic.

The first clinical trial carried out using the Mahidol tetravalent vaccine in US volunteers revealed that antibody responses were predominantly directed against DEN3 with low or undetectable titres against the remaining three serotypes [6]. This outcome has been attributed to preferential replication of DEN3 in the tetravalent vaccines. A subsequent clinical study in Thailand showed that varying and reducing the concentrations of the DEN3 strain resulted in improved clinical safety profile of the tetravalent vaccine. After a single dose, 58% of the recipients seroconverted against three or more serotypes and 35% seroconverted against all four. After the second dose, seroconversion was 76% and 71%, respectively. All subjects seroconverted to DEN3 after one dose. DEN4 elicited the lowest primary response but the highest increase in seroconversion after the second dose [7]. Phase 3 trials are underway where the vaccine is being tested against 20,000 children.

Another dengue vaccine has been developed by the

US Armed Forces at Walter Reed Army Institute of Research (WRAIR). All four monovalent WRAIR live attenuated vaccines elicited seroconversions in human volunteers (DEN1= 100%; DEN2=92%; DEN3=46%; DEN4= 58%). Tetravalent formulations have been prepared and analysed by pre-clinical testing in rhesus monkeys and Phase 1 and 2 clinical trials in humans. Vaccination-challenge studies in rhesus monkeys using a tetravalent formulation demonstrated that most animals seroconverted (DEN1= 100%, DEN2=100%; DEN3=90%; DEN4=70%) after two doses of the vaccine [8]. In pilot human studies (n=10) three doses were required to achieve a seroconversion rate of 50% to all four serotypes. Seroconversion rates for dose-optimised tetravalent vaccine approached that of the monovalent formulations (DEN1=94%; DEN2=76%; DEN3=70%; DEN4=47%, n=37) [9]. Phase 2 trials of the vaccine are conducted in Thailand and Panama.

Although the live, attenuated vaccine has been found to be safe and immunogenic, questions remain regarding reversion of the vaccine virus to a virulent form of the dengue virus.

Intertypic Chimaeric Vaccines

In this approach, the structural genes from the cDNA copy of an attenuated strain of dengue virus of a given serotype is replaced by the corresponding genes of a different dengue virus serotype. Intertypic chimaeric dengue viruses have been made containing the nonstructural genes of DEN4, and the structural genome of either DEN1 or DEN2. The resultant intertypic chimaeric vaccines elicit antibody responses specific to the serotype from which their structural genes are derived.

Rhesus monkeys, when immunized with intertypic Chimaeric DEN1/DEN4 vaccine, developed neutralising antibodies against DEN1 and were protected against subsequent DEN1 challenge. Similarly, monkeys immunised with DEN2/DEN4 developed antibodies against DEN2 and were protected against subsequent

Table 1
Current status of dengue vaccine development

Vaccine Type	Developed By	Country	Clinical Trials	Licensed to
Live attenuated tetravalent vaccine	Mahidol University	Thailand	In Phase III	Sanofi Pasteur
Live attenuated tetravalent vaccine	WRAIR*	United States	In Phase II	GlaxoSmithKline Biologicals
Intertypic chimaeric vaccine	NIAID, NIH*	United States	In Phase II	Not licensed
Chimaeric vaccine	Acambis	United States	In Phase II	Sanofi Pasteur
Chimaeric vaccine	CDC*	United States	In Phase I	Inviragen
Flavivirus-based recombinant DNA vaccine	Navy Medical Research Center	United States	In Phase I	Not licensed
Non-flavivirus based recombinant DNA vaccine	Hawaii Biotech	United States	Preclinical	Not licensed

* Walter Reed Army Institute of Research (WRAIR); National Institute of Allergy and Infectious Diseases (NIAID); National Institutes of Health (NIH); Centers for Disease Control and Prevention (CDC)

DEN2 challenge. DEN1- and DEN2-immunised monkeys were protected against homologous virus challenge, but DEN4-immunised animals became viraemic on cross-challenge with DEN1 or DEN2 [10].

In a second experiment, monkeys immunised with equal mixtures of DEN1/DEN4 and DEN2/DEN4 developed neutralising antibodies against both DEN1 and DEN2 and were protected against subsequent challenge with DEN1 or DEN2. The high degree of immunogenicity and very mild reactogenicity of the mutant virus have prompted efforts to use it as a vector for the construction of chimaeric viruses encoding the structural proteins of DEN1, DEN2 and DEN3.

Current initiatives that seek to develop intertypic chimaeric vaccines are focusing on the use of attenuated strains of DEN1, DEN2 and DEN4 as vectors to carry heterotypic structural genes. The structural genes from a cDNA copy of the Mahidol vaccine candidate DEN2 PDK-53 have been replaced with the corresponding genes of DEN1 using infectious clone technology [11]. A DEN4 mutant bearing a 30-nucleotide (nt) deletion in its 3' untranslated region has been tested in 20 human volunteers who exhibited only minor symptoms with 100% neutralising antibody seroconversion [12].

Chimaeric Vaccines

The ChimaeriVaxTM system, first used to develop a candidate live attenuated Japanese Encephalitis (JE) vaccine, has now been applied to dengue viruses in the United States. This approach replaces the E gene of the 17D yellow fever (YF) vaccine with the analogous gene of the vaccine-targeted flavivirus [13]. Chimaeric YF/DEN viruses have been constructed for all four serotypes, utilising the donor genes from DEN1, PUO-359; DEN2, POU-218; DEN3, PaH881; and DEN4, 1228 strains. The only difference with respect to the intertypic strategy is that the ChimaeriVax approach is based on the use of the attenuated YF17D vector rather than attenuated dengue virus vectors.

All YF/DEN chimaeras grow to high titre in cell culture and show lower neurovirulence following intracerebral inoculation in mice than the parent YF 17D vaccine. All four DEN/YF chimaeras produce short-lived, low level viremia in rhesus monkeys following subcutaneous inoculation and elicit a dose dependent virus neutralising antibody response.

Immunisation of monkeys with a tetravalent formulation (mixture of equal concentrations of each monovalent chimaeric virus) of the YF/DEN chimaeric vaccine resulted in the highest immune response against the YF/DEN-2 virus. A dose adjustment for the YF/DEN2 chimaera resulted in a more balanced response against DEN1, DEN2 and DEN3 viruses, but a

somewhat higher response against the chimaeric DEN4 virus [14]. Phase 1 trials of a tetravalent formulation of chimaeric vaccines among healthy adult volunteers have been completed. Results from the trial have shown seroconversion to all four dengue virus serotypes. The vaccine has now progressed into Phase 2 clinical trials.

There does not appear to be any interference between YF and DEN/YF chimaeric vaccines in the respective immune responses, nor are the chimaeric vaccines able to replicate in mosquitoes. The high replication efficiency, immunogenicity, protective efficacy and genomic stability of chimaeric vaccines justify them as novel candidate vaccines for use in humans in the near future.

Flavivirus-based Recombinant Vaccines

Infectious clone technology is being used for the development of a dengue vaccine which permits recovery of the infectious dengue virus from cells transfected *in vitro* with RNA transcripts derived from a full length cDNA clone of the dengue virus genome. Using this strategy, infectious recombinant viruses have been created from cDNA clones engineered to carry defined attenuating mutations. It is also possible to produce monovalent chimaeric viruses by replacing the structural genes of the full-length cDNA clone with those of different dengue virus serotypes. These vaccines focus on the E protein. Most vaccine designs also include the prM protein, which has been implicated in maintenance of the structural/antigenic integrity of the E protein [15].

Non-flavivirus based Recombinant Vaccines

Vectors derived from baculovirus and vaccinia virus are being used as heterologous viral vector systems for the expression of dengue structural antigens. However, the use of baculoviruses as live viral vaccine vectors does not appear to be currently feasible due to their inactivation mediated by complement.

Recombinant vaccinia vectors harbouring different flavivirus genes have been tested as vaccines in animal models. Mice and monkeys immunised with a recombinant vaccinia vector encoding DEN4 structural proteins prM and E, and the non-structural protein NS1 were found to be protected [16]. However, safety concerns exist with the use of vaccinia virus-vectored vaccines because most of the recombinant vaccinia vectors designed to express dengue antigens are based on the virulent WR strain, which are unacceptable for human use.

To tackle these complexities, the highly attenuated modified vaccinia virus Ankara (MVA) has been used as a potential dengue vaccine vector. MVA does not

replicate efficiently in human cells and this character is genetically stable. A recent study showed that monkeys repeatedly immunised with MVA recombinant expressing DEN2 E protein had virus-neutralising antibodies and were fully protected against challenge with homotypic dengue virus [17].

Paediatric Dengue Vaccine Initiative

The Paediatric Dengue Vaccine Initiative (PDVI) was established in 2003 at the International Vaccine Institute in Seoul, South Korea, with a mission to accelerate evaluation of candidate dengue vaccines, introduction of improved diagnostics and to introduce affordable safe vaccines in dengue endemic countries.

PDVI is also serving as a forum for improved advocacy and coordination of global efforts on dengue vaccine development. Till date, PDVI has received financial support totalling \$60 million from the Rockefeller Foundation, the Bill and Melinda Gates Foundation and the World Health Organisation [18].

Conclusion

Continued progress on vaccine development has increased the likelihood that an effective vaccine against dengue is technically possible, with most of the vaccines reaching the final stages of development [19]. Efforts are continuing to optimise the monovalent vaccines into a single tetravalent package.

A concerted effort by all stakeholders is required to overcome the many obstacles including the cost factor before a dengue vaccine can be licensed. The generous funding received by the PDVI could act as a catalyst towards this endeavour. Although the greatest morbidity and mortality from dengue occurs amongst children living in developing countries including India, current dengue vaccines are primarily targeted towards adult travellers and western military personnel.

Once a dengue vaccine is close to licensure, cost-effective analysis comparing vaccination costs with expenditures for treatment and control of dengue would assist the Indian government and the Armed Forces in setting health priorities and making crucial decisions on dengue vaccination.

Conflicts of Interest

None identified

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