

Viral Vaccines for Dengue: The Present and the Future

by

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Abstract

Infection with dengue viruses, of which there are four antigenically distinct serotypes, has re-emerged as a significant global public health threat. Sequential infection in areas of hyperendemicity, where multiple serotypes co-circulate, has the potential to trigger life-threatening disease. Therefore, a safe and effective dengue vaccine must be 'tetravalent' capable of providing solid and long-lasting immunity to all four serotypes. The development of a vaccine against dengue has been a high priority of the World Health Organization (WHO) for decades. Currently, six different virus-based vaccines are in various stages of development. Two of these are traditional tissue culture-based live attenuated vaccines whereas the remaining four are chimeric recombinant vaccine viruses developed using infectious clone technology. In all these instances, the vaccine viruses are monovalent in that each one is specific to one dengue serotype. A tetravalent dengue vaccine is based on producing vaccine formulations by mixing all four monovalent vaccine viruses. Recent studies in both monkeys and man have shown that such tetravalent formulations can elicit unbalanced immune responses due to the phenomenon of viral interference. While efforts are under way to optimize the tetravalent formulations, the inherent risk of viral interference associated with the current strategy of producing tetravalent dengue vaccine warrants investigation of other recombinant viral systems that eliminate this risk, especially the replication-defective adenovirus-based vector system that may permit the creation of a single vaccine vector capable of conferring tetravalent protection.

Keywords: Dengue virus, dengue vaccine, adenovirus.

Introduction

Dengue viruses are mosquito-borne, positive-stranded RNA viruses of the genus *Flavivirus*. These are human pathogens with a worldwide prevalence. About 2.5 billion people in more than a hundred countries are

estimated to be at risk of dengue virus infection, with millions of cases occurring every year around the world⁽¹⁾. There are four antigenically distinct serotypes of dengue viruses (DEN-1, 2, 3 and 4), which can cause a broad spectrum of illness ranging from mild febrile sickness to severe

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haemorrhagic, sometimes fatal, disease. To date, there is no licensed vaccine to prevent dengue infection.

Developing a vaccine for dengue is a very challenging task because of two important reasons. First, dengue infections can be more severe in individuals who have dengue antibodies acquired either passively through maternal transmission or actively from a prior episode of dengue infection with a heterologous serotype⁽²⁾. Thus, protection against only one or two dengue viruses could actually increase the risk of potentially fatal dengue haemorrhagic fever and dengue shock syndrome through antibody dependent enhancement (ADE) of infection. Thus, a successful vaccine must be 'tetravalent', capable of simultaneously inducing a high level of long-lasting immunity to all four serotypes. Secondly, a suitable animal model to evaluate candidate dengue vaccines is not available. These setbacks notwithstanding, many laboratories worldwide are exploring multiple approaches towards developing dengue vaccines. A major part of current research efforts on dengue vaccine development focuses on the use of live-attenuated and infectious clone-derived vaccines^(3,4). Various laboratories are engaged in exploring other alternative strategies, including DNA vaccines⁽⁵⁾, protein vaccines and immunogens vectored by various recombinant systems⁽⁶⁾.

The provision of an effective means to stimulate both the humoral and cellular arms of the immune response is crucial to vaccine development. Protein-based vaccines elicit potent antibody responses

(humoral response) while failing to elicit an effective cellular response. In contrast, DNA- and virus-based vaccines can elicit both humoral and cellular immune responses. However, DNA-based vaccines tend to induce relatively low antibody titres, presumably due to the poor efficiency of target cell transduction and concomitant low-level antigen expression⁽⁷⁾. Induction of low titres of antibodies is potentially risky in the context of ADE. Virus-based vaccines are decidedly more effective in this regard as they can mediate efficient antigen gene transfer and high-level production of authentic protein antigens directly within the cells of the immunized host in the natural context of an ongoing infection and thus provide for a balanced stimulation of both arms of the immune system and resultant long-lasting immunity⁽⁸⁾. Recent investigations using virus-based recombinant and non-recombinant dengue vaccine candidates in monkeys^(9,10) and man^(11,12), respectively, have shown that the current strategy of creating tetravalent dengue vaccine formulations can lead to an unbalanced immune response, specific to only one particular serotype. This has been attributed to 'viral interference' that apparently comes into play when four monovalent vaccine viruses are mixed to create a tetravalent formulation. This article reviews the current status of these vaccine candidates and examines the potential of alternate viral vector systems that might eliminate the phenomenon of viral interference and pave the way towards achieving a balanced immune response against all four dengue virus serotypes.

Conventional vaccines

Empirically attenuated strains of all four dengue serotypes have been created by repeated serial passage in non-permissive cell lines independently by Mahidol University in Thailand and the Walter Reed Army Institute for Research (WRAIR) in USA^(3,4). In both cases physical mixtures of the four different attenuated strains are prepared to produce tetravalent formulations. The Mahidol vaccine has been licensed to Aventis Pasteur (AvP) in France for large-scale production under Good Manufacturing Practice (GMP) conditions. The first clinical trial carried out using the Mahidol/AvP tetravalent vaccine in US volunteers revealed that antibody responses were predominantly directed against DEN-3 with low or undetectable titres against the remaining three serotypes⁽¹¹⁾. This outcome has been attributed to preferential replication of DEN-3 in the tetravalent vaccines. The mechanism of such viral interference is not known. But, it has been suggested that the ratio of the four attenuated viruses in the tetravalent formulation may be an important factor. A subsequent clinical study in Thailand⁽¹²⁾ showed that varying and reducing the concentrations of the serotype 3 strain resulted in improved clinical safety profile of the tetravalent vaccine. About 71% seroconversion (against all four serotypes) was observed after a two-dose vaccination schedule in this study. Several different reformulations of the tetravalent vaccine are being evaluated in order to get a more balanced immune response to each serotype. The WRAIR vaccine, licensed to GlaxoSmithKline in Belgium, has recently been quoted to have been tested in a phase

I study of 50 adult volunteers. It has been further quoted that this study, which evaluated 16 different formulations of the tetravalent vaccine, reported an 80-90% seroconversion against all four serotypes after two immunizations^(3,4).

Flavivirus-based recombinant dengue vaccines

A different approach for creating viral vaccines against dengue has taken advantage of the infectious clone technology. This technology permits the recovery of 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 can be created from cDNA clones engineered to carry defined attenuating mutations. It is also possible to produce monovalent chimeric viruses by replacing the structural genes of the full-length cDNA clone with those of different DEN virus serotypes. Infectious clone technology-based approaches (as well as other recombinant virus vectored vaccines, see below) have focused on the dengue virus envelope (E) protein (the major structural protein), for a variety of reasons^(13,14,15,16,17,18) summarized in Table 1, the most important ones being that it mediates virus entry by interacting with host cell surface receptors⁽¹³⁾ and is the primary target of the neutralizing antibody response^(14,15). Most vaccine designs also include another structural protein, the premembrane (prM) protein, implicated in the maintenance of the structural/antigenic integrity of the E protein⁽¹⁹⁾.

Table 1. Most recombinant dengue vaccine efforts focus on the E protein

Properties/functions of the E protein	References [¶]
• Binds host cell surface receptor	Chen <i>et al</i> , 1996 ⁽¹³⁾
• Target of neutralizing antibodies	Mégret <i>et al</i> , 1992 ⁽¹⁴⁾
• Elicits the first antibody response with the longest-lasting activity	Churdboonchart <i>et al</i> , 1991 ⁽¹⁵⁾
• Several monoclonal antibodies specific to receptor binding domain of E protein block virus adsorption and infectivity	Crill and Roehrig, 2001 ⁽¹⁶⁾
• Anti-E monoclonal antibodies can confer passive protection	Kaufman <i>et al</i> , 1987 ⁽¹⁷⁾
• Purified E protein can induce neutralizing antibodies and protective immunity	Feighny <i>et al</i> , 1992 ⁽¹⁸⁾

[¶] Reference numbers are shown in parentheses

Intertypic chimeric vaccines

In this approach, pioneered by Lai's group at the National Institutes of Health, Bethesda, USA, 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. The resultant intertypic chimeras are monovalent in that they elicit antibody responses specific to the serotype from which their structural genes are derived⁽²⁰⁾. Current initiatives that seek to develop intertypic vaccine viruses are focusing on the use of attenuated (either by conventional methods or by site-directed mutagenesis) strains of DEN-1^(21,22), DEN-2^(23,24) and DEN-4⁽²⁵⁾ as vectors to carry heterotypic structural genes (see Table 2).

For example, the structural genes from a cDNA copy of the Mahidol vaccine candidate DEN-2 PDK-53 (attenuated by repeated serial passaging in primary dog kidney cells and tested in US and Thai volunteers) have been replaced with the corresponding genes of DEN-1 using infectious clone technology⁽²³⁾. A DEN-4 mutant bearing a 30-nucleotide (nt) deletion in its 3' untranslated region (UTR) has been tested in 20 human volunteers who exhibited only minor symptoms with 100% neutralising antibody seroconversion⁽²⁵⁾. The high degree of immunogenicity and very mild reactogenicity of this mutant virus have prompted efforts to use it as a vector for the construction of chimeric viruses encoding the structural proteins of DEN-1, DEN-2 and DEN-3. More recently, this 30-nt deletion has also been introduced into the 3' UTR of wild-type DEN-1 virus with resultant attenuation⁽²²⁾. This finding opens up the possibility that a tetravalent vaccine could be generated by the introduction of this 30-nt deletion into wild-type dengue viruses of all four serotypes.

Table 2. Attenuated dengue virus strains for development of intertypic chimeric vaccines

Vaccine strain (parent virus)	Location of attenuating mutations	Attenuation strategy	References [¶]
rDen1mutF (DEN-1)	3' SL ^ψ	site-directed mutagenesis	Markoff <i>et al</i> , 2002 ⁽²¹⁾
rDen1? 30 (DEN-1)	3' UTR	site-directed mutagenesis	Whitehead <i>et al</i> , 2003 ⁽²²⁾
PDK-53 (DEN-2)	5'UTR NS1, NS3	serial passage in PDK cells	Huang <i>et al</i> , 2000 ⁽²³⁾ Butrapet <i>et al</i> , 2000 ⁽²⁴⁾
2A? 30 (DEN-4)	3'UTR	site-directed mutagenesis	Durbin <i>et al</i> , 2001 ⁽²⁵⁾

[¶] Reference numbers are shown in parentheses

^ψ Abbreviations: SL, stem-loop; UTR untranslated region; NS1 and NS3, non-structural proteins 1 and 3; PDK, primary dog kidney cells

ChimeriVax vaccines

The most successful flavivirus vaccine is the empirically attenuated 17D strain of yellow fever (YF) virus⁽²⁶⁾. A single immunization with the YF17D vaccine strain can confer immunity against yellow fever in nearly 100% of vaccinated individuals. Using the YF17D vector, Acambis, USA, has developed a promising platform, known as ChimeriVax technology, for the development of dengue vaccines. In this strategy, the structural genes (prM and E) of YF17D in a full-length cDNA clone are replaced with the corresponding genes from dengue viruses. The only difference with respect to the intertypic strategy is that the ChimeriVax approach is based on the use of the attenuated YF17D vector rather than attenuated dengue virus vectors. Acambis has created four monovalent chimeric vaccine viruses using this technology (YF/DEN-1 to DEN-4), each encoding the structural genes of one of the four dengue serotypes⁽⁹⁾ and licensed these to Aventis Pasteur, France. Immunization of

monkeys with a tetravalent formulation (physical mixture of equal concentrations of each monovalent YF/DEN chimeric virus) of the YF/DEN chimeric vaccine resulted in the highest immune response being directed against the YF/DEN-2 virus, a situation reminiscent of that noted earlier for the live attenuated Mahidol/AvP vaccine⁽¹¹⁾. A dose adjustment for the YF/DEN-2 chimera resulted in a more balanced response against DEN-1, DEN-2 and DEN-3 viruses, but a somewhat higher response against the chimeric DEN-4 virus⁽¹⁰⁾. Additional formulations will have to be evaluated in monkeys before undertaking Phase I human trials.

Recombinant dengue vaccines based on non-flavivirus vectors

Apart from the use of attenuated flaviviruses derived from different serotypes of dengue

viruses and YF virus, efforts are on to use several well-characterized heterologous viral vector systems for the expression of dengue structural antigens. Prominent among these are vectors derived from baculovirus⁽²⁷⁾ and vaccinia virus⁽²⁸⁾. Recent work suggests that baculoviruses can transduce a variety of mammalian cells; however, their use as live viral vaccine vectors does not appear to be currently feasible due to their inactivation mediated by complement⁽²⁹⁾. This section will review work that pertains to the use of vaccinia virus vectors which have been tested as potential candidate dengue vaccines in animal models and adenovirus vectors, which have not been evaluated for the expression of dengue antigens so far, despite their tremendous potential to serve as safe and efficacious viral vaccine vectors⁽³⁰⁾.

Vaccinia virus vectors

Recombinant vaccinia vectors harbouring different flavivirus genes have been constructed and tested as vaccines in animal models during the late eighties and early nineties^(31,32,33,34). Mice immunized with a recombinant vaccinia vector encoding DEN-4 structural proteins prM and E, and the non-structural protein NS1 (either singly or in combination) were protected against lethal dengue encephalitis⁽³¹⁾. Despite the observed protection, only low levels of E-specific neutralizing antibodies were detected. The vaccinia expressed DEN-4 E was found to be poorly immunogenic in monkeys as well⁽³²⁾. In a subsequent report, it was shown that a vaccinia recombinant, which expressed a carboxy-terminally truncated version of DEN-4 E, was more immunogenic than

recombinants expressing full-length DEN-4 E⁽³³⁾. Co-expression of prM and E using vaccinia vectors can lead to the secretion of extracellular sub-viral particles capable of eliciting high levels of neutralizing antibodies⁽³⁴⁾. However, safety concerns exist with the use of vaccinia virus-vectored vaccines because of the undesirable side-effects demonstrated during the WHO-directed smallpox eradication programme. As most of the recombinant vaccinia vectors designed to express dengue antigens have been based on the virulent WR strain, these are unacceptable for human use⁽³⁵⁾. These concerns have led, in the last few years, to the use of a highly-attenuated vaccinia virus vector called modified vaccinia virus Ankara (MVA) as a potential dengue vaccine vector. A recent study⁽²⁸⁾ which investigated the use of MVA to express the carboxy-terminally truncated versions of the E proteins of DEN-2 and DEN-4 showed that mice immunized with MVA-DEN-2E, but not MVA-DEN-4E, induced a neutralizing antibody response. Furthermore, it was shown that MVA-vectored DEN-2E could protect monkeys against the DEN-2 virus challenge⁽²⁸⁾. Though the safety of the MVA virus in humans has been well-documented, it appears to be associated with inconsistent antigen-specific immune responses⁽³⁶⁾.

Adenovirus vectors

The feasibility of using adenovirus (Ad)-based vaccine vectors for expressing dengue antigens has not been explored so far, despite its many advantages^(8,30,37,38,39,40,41,42,43,44,45). The salient features of Ad vectors are summarized in Table 3.

Table 3. Adenovirus has several features suitable for its development as a tetravalent dengue vaccine vector

Features of Ad vectors	References [¶]
Well-documented human safety	Gaydos & Gaydos, 1999 ⁽³⁷⁾
No significant pathology	Horwitz, 2001 ⁽³⁸⁾
Large insert capacity (7.5-35Kb)	Wang & Huang, 2000 ⁽³⁹⁾
Oral immunization	Top <i>et al</i> , 1971 ^(40, 41)
Can elicit humoral, cell-mediated and mucosal immunity	Imler, 1995 ⁽⁴²⁾ Rolph and Ramshaw, 1997 ⁽⁸⁾
Replication-defective Ad is superior to poxvirus vectors	Gonin <i>et al</i> , 1996 ⁽⁴³⁾ Xiang <i>et al</i> , 1996 ⁽⁴⁴⁾ Shiver <i>et al</i> , 2002 ⁽⁴⁵⁾

[¶] Reference numbers are shown in parentheses

A major factor in favour of exploring Ad-vectored dengue vaccine candidates is Ad's safety record. Live Ad vaccines have been successfully and safely administered orally to millions of US military recruits⁽³⁷⁾. Ad is not associated with any significant pathology in humans⁽³⁸⁾. It can be propagated to very high titres of about 10¹¹⁻¹² plaque forming units (pfu)/ml^(42,46), (unlike flaviviruses which yield titres of only around 10⁶⁻⁷ pfu/ml), in permissive cell lines and can be purified relatively easily using column chromatographic methods⁽⁴⁷⁾. A number of viral antigens derived from several different viruses have been expressed using Ad-based vectors. These recombinant Ad vectors have been tested in several animal models and have been shown to elicit humoral, cell-mediated and mucosal immunity.

Furthermore, protection against virus challenge has been demonstrated in a number of experimental models^(8,42,43,45,48). Two of the most promising recent reports pertaining to non-human primate models of the Ebola virus⁽⁴⁸⁾ and the human immunodeficiency virus⁽⁴⁵⁾ emphasize the potential of Ad-based vaccine strategy. Comparison with attenuated poxvirus vectors such as New York vaccinia (NYVAC)⁽⁴³⁾ and MVA⁽⁴⁵⁾ have shown Ad to be safer and more efficacious. Our preliminary work has demonstrated that a recombinant Ad expressing the DEN-2E protein is capable of eliciting antigen-specific neutralizing antibodies⁽⁴⁹⁾. The most widely used Ad vectors are all replication-defective. This will ensure that Ad will not disseminate in the vaccinee, thus minimizing the risk of adverse side-effects⁽³⁰⁾. As Ad is a ubiquitous virus, a potential disadvantage with the use of Ad-based vaccines is the issue of prior Ad immunity. However, this may no longer be a serious concern. Co-injection of gel foam (a collagen-based matrix) with the antigen-encoding Ad vaccine virus has been demonstrated to abrogate the inhibitory effects of Ad immunity⁽⁵⁰⁾. A more recent report has shown that potential interference by pre-existing immunity to the viral vaccine can be effectively overcome by DNA priming before vector boosting⁽⁵¹⁾.

Conclusions

Dengue infection has emerged as a leading public health concern in over a hundred tropical and sub-tropical countries in south-east Asia, the Caribbean and Central and South Americas. A vaccine is urgently needed to lessen the global dengue disease

burden. No vaccine is currently licensed for human use. Developing a dengue vaccine has been an elusive goal because of the need to confer solid and long-lasting tetravalent protection and the lack of a good animal model in which to evaluate the experimental vaccines. Dengue vaccine research focuses on several strategies. The major focus is on the use of live-attenuated and infectious clone-derived vaccines. Currently, six different virus-based vaccines are in advanced stages of development. Two of these are traditional tissue culture-based live attenuated vaccines, whereas the remaining four are chimeric recombinant vaccine viruses developed using infectious clone technology. In all these instances, the vaccine viruses are monovalent in that each one is specific to one dengue serotype. A tetravalent dengue vaccine is based on producing vaccine formulations by mixing all four monovalent vaccine viruses. Recent studies have shown that both the live attenuated⁽¹¹⁾ and the ChimeriVax⁽¹⁰⁾ tetravalent dengue vaccine formulations elicit unbalanced immune response due to viral interference. The occurrence of this phenomenon, which tends to skew the immune response predominantly towards one serotype (especially in the case of the ChimeriVax dengue vaccine in which all the four monovalent vaccine viruses have

identical backbones), emphasizes the limitations and, more importantly, the risks associated with mixing four monovalent vaccine viruses to create a tetravalent vaccine.

While efforts are under way to optimize the tetravalent formulations, the risk of viral interference inherent in the current strategy of producing tetravalent dengue vaccine warrants investigation of other recombinant viral vector systems that may permit the creation of a single vaccine vector capable of conferring tetravalent protection. Promising progress in the development of alternative vaccine strategies, particularly those using live viral vectors, have the potential to be developed into single component vaccines incorporating all the key antigens from all four DEN serotypes. Viral vectors derived from vaccinia virus and adenovirus have fairly large insert capacities and are potential candidates for exploring the possibility of developing such a single tetravalent vaccine vector. While the use of poxvirus vectors is associated with safety concerns⁽³⁵⁾, replication-defective adenoviruses are not associated with any significant human pathology⁽³⁸⁾. The large insert capacity, ranging from 7.5-35 kb⁽³⁹⁾, its ability to grow to very high titres (10^{11-12} pfu/ml) and the ease of scale-up and purification⁽⁴⁶⁾, confer on Ad the potential to be developed into a cost-effective tetravalent dengue vaccine.

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