Latest developments and future directions in dengue vaccines

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Abstract: Dengue is a mosquito-borne disease which is currently an expanding global health problem. The disease is caused by four closely related viruses, the dengue virus. There are no specific dengue therapeutics and prevention is currently limited to vector control measures. Development of an effective tetravalent dengue vaccine would therefore represent a major advance in the control of the disease and is considered a high public health priority. While a licensed dengue vaccine is not yet available, the scope and intensity of dengue vaccine development has increased dramatically in the last decade. The uniqueness of the dengue viruses and the spectrum of disease resulting from infection have made dengue vaccine development difficult. Several vaccine candidates are currently being evaluated in clinical studies. The candidate currently at the most advanced clinical development stage, a live-attenuated tetravalent vaccine based on chimeric yellow fever dengue virus, has progressed to phase III efficacy studies. Several other live-attenuated vaccines, as well as subunit, DNA and purified inactivated vaccine candidates, are at earlier stages of clinical development. Additional technological approaches, such as virus-vectored and virus-like particle-based vaccines, are under evaluation in preclinical studies.

Keywords: dengue infection, dengue fever, dengue vaccines, *Flavivirus*, live-attenuated tetravalent vaccine, development

Introduction

Dengue infection, one of the most devastating mosquito-borne viral diseases in humans, is now a significant problem in many countries. The causative dengue viruses are members of the genus *Flavivirus*, within the family *Flaviviridae*. There are four closely related serotypes, the dengue viruses (DENV) 1–4 and at least four genotypes within each serotype. Primary infection with a particular dengue serotype confers long-lasting immunity for that serotype (homotypic immunity). Immunity to other dengue serotypes (hetrotypic immunity) lasts for a few months, after which patients are susceptible to heterotypic infection. All flaviviruses are lipid-enveloped, positive-strand RNA viruses. The RNA genome of dengue virus is about 10.7 kb and encodes three structural proteins, namely capsid protein (C), precursor membrane/membrane protein (PrM/M) and envelope protein (E). Besides the structural proteins, there are seven nonstructural proteins (NS) which are associated with viral replication and disease pathogenesis. The coding of the viral proteins is organized in the genome as C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5. The disease, caused by the four dengue virus serotypes, ranges from asymptomatic infection to undifferentiated fever, dengue fever (DF), and severe dengue hemorrhagic fever (DHF). DHF is characterized by fever, bleeding diathesis and plasma leakage with a tendency to develop a potentially fatal shock syndrome. Dengue infection with organ impairment mainly involves the central nervous system and liver. Consistent hematological findings include vasculopathy, coagulopathy, and thrombocytopenia. Laboratory diagnosis includes virus isolation, serology, and detection of dengue ribonucleic acid. Successful treatment, which is mainly supportive, depends on early recognition of the disease and careful monitoring for shock. A severity-based revised dengue classification for medical interventions has been developed and validated in many countries.

Prevention depends primarily on control of the mosquito vector which has achieved only limited success in reducing transmission of dengue.

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The primary vector, the urban-adapted *Aedes aegypti*, has become widely distributed across tropical and subtropical latitudes. It has spread globally with the advent of increased travel and the trade in the past 50 years. A secondary vector, *Aedes albopictus*, has expanded dramatically in recent years. The reasons for continued transmission are population growth, rapid urbanization, environmental change, climate change, modern transportation, inadequate water storage, lack of political commitment, and lack of intersectoral collaboration. The pathogenesis of DHF is not clearly understood. One hypothesis concerning virus virulence has been debated with the immune enhancement hypothesis. Although dengue disease severity has been associated with evidence of genetic differences in dengue strains, virus virulence has been difficult to measure because of the lack of *in vivo* and *in vitro* models of disease. Dengue is a serious and growing threat to public health in Southeast Asia which bears nearly 75% of the current global dengue burden. Specific antiviral medications are not available for dengue and prevention using vector control has had some but limited success. Vaccines for dengue are in clinical development, with the lead candidate currently in phase III clinical trials. Dengue vaccine may be the major mean to effectively control dengue diseases [Hemungkorn *et al.* 2007; Wan *et al.* 2013].

Current development of dengue vaccines

The first dengue vaccines were evaluated in 1929 [Blac and Caminopetros, 1930; Simmons *et al.* 1931]. Development of safe and effective dengue vaccines faces many challenges. Infection by one of the four dengue virus serotypes has been shown to confer lasting protection against homotypic reinfection, but only transient protection against a secondary heterotypic infection. Moreover, secondary heterotypic infection is associated with an increased risk of severe disease. This and other observations suggest an immunopathological component in dengue pathogenesis, which is referred to as immune enhancement of disease. Due to these dengue-specific complexities, vaccine development focuses on the generation of a tetravalent vaccine aimed at providing long-term protection against all virus serotypes. In theory, a tetravalent immune response would protect against dengue febrile illness and would also reduce or eliminate the risk of antibody-dependent enhancement which is thought to be one of the mechanisms that predispose people to severe dengue. Additional challenges are posed by the lack of an adequate animal disease model and the resulting uncertainty around correlates of protection. In spite of these challenges, vaccine development has made remarkable progress in recent years, and the current dengue vaccine pipeline is advanced, diverse, and overall promising.

Tetravalent dengue vaccine must be developed without the benefit of a full understanding of the pathogenesis of severe dengue disease or an adequate animal disease model [Prommalikit *et al.* 2004; Prommalikit and Thisyakorn, 2013]. In the last decade dengue vaccines development efforts have increased dramatically due to an increased awareness of the dengue pandemic and the development of new molecular techniques.

There are several important safety issues for dengue vaccines. Principal among these concerns is the theoretical risk of enhanced disease following dengue vaccination. The rationale for a tetravalent vaccine is the perceived requirement to induce primary-type immune responses to all four dengue viruses simultaneously. The simultaneous production of immune response specific to each of the four dengue viruses is predicted to minimize the risk of disease enhancement following natural infection. However, antibody-dependent enhancement may occur with neutralizing antibodies at subneutralizing concentrations, so a vaccine that induces protection for a period of time might later increase the risk for enhanced disease. This is particularly a concern for vaccines that induce low levels of neutralizing antibodies [Burton *et al.* 2000], but might occur with any vaccine given enough time. To adequately assess this risk and the risk of incomplete immunization and waning antibody titers, dengue vaccine clinical development plans must include flavivirus-primed and flavivirus-naïve volunteers, and sufficiently long-term follow up to make statistically powered conclusions regarding the safety of dengue vaccination in flavivirus-endemic areas. Other safety concerns with live attenuated virus vaccines include cell-culture-derived adventitious agents, community spread of the vaccine virus by resident vector mosquitoes, vaccine virus neurovirulence, and the effects of vaccine administration to immunocompromised hosts. Although these safety concerns are theoretically possible, they are extremely unlikely with the current evidence [Edelman and Hombach, 2008].

At present, several dengue vaccines have been tested in human clinical trials, and a single

candidate is now in phase III clinical trials. Different approaches in dengue vaccine development are discussed herein.

Live attenuated virus

The first major effort at live attenuated dengue vaccine development began at the University of Hawaii using the traditional method of serial passage of virus in a nonhuman host and then transferred to Mahidol University in Bangkok, Thailand for further passage and development of candidate vaccines and testing [Bhamarapravati and Sutee, 2000; Halstead and Marchette, 2003]. The candidate vaccine was used for phase I and II clinical trials in Thai adults and children. Not all of the volunteers seroconverted to all four dengue serotypes and some showed unacceptable reactogenicity, consequently further clinical testing was stopped [Sabchareon *et al.* 2002, 2004; Sanchez *et al.* 2006]. Although the development of this candidate vaccine was not successful, the initiative was responsible for the subsequent progress that has been made in developing a live attenuated tetravalent dengue vaccine [World Health Organization, 2010].

The second tissue-culture-passaged dengue vaccine was developed at the Walter Reed Army Institute of Research (WRAIR). The WRAIR-produced tetravalent dengue vaccine initial formulation also showed problems of unbalanced immunogenicity and reactogenicity [Sun *et al.* 2003].

New formulations seem to be safe and immunogenic in a phase II study, however the protective efficacy needs to be further evaluated [Thomas *et al.* 2013]. Further testing is delayed because of manufacturing complexities and determination of the optimal dose and schedule of vaccine [Balas *et al.* 2011; Halstead and Thomas, 2013].

The US National Institutes of Health introduced a new era of dengue vaccine research with direct mutagenesis technology. Dengue genomes were readily altered genetically, resulting in attenuated variants which have been tested in flavivirus-naïve US adults. The National Institutes of Health has licensed the vaccine candidates to several institutions for further testing.

The US Food and Drug Administration also created molecularly attenuated tetravalent dengue vaccine which has been tested in nonhuman primates [Lai *et al.* 1991; Polo *et al.* 1997; Halstead and Thomas, 2013].

Both techniques provide an alternative approach to constructing a live attenuated tetravalent dengue vaccine [Halstead and Thomas, 2013].

Chimeric virus

The US Centers for Disease Control and Prevention (CDC) developed a tetravalent chimeric dengue vaccine by inserting DENV-1, -3 and -4 prM and E genes into cDNA derived from the successfully attenuated DEN-2 component of the Mahidol University-Sanofi Pasteur live attenuated dengue virus vaccine (DEN-2, 16681 PDK-53).

Dengue-dengue chimeras tetravalent vaccine candidate was then formulated and licensed to Inviragen, Inc. and Takeda respectively and has undergone clinical testing [Lai *et al.* 1991; Polo *et al.* 1997; Halstead and Thomas, 2013; Huang *et al.* 2013].

A different approach was taken to insert dengue structural genes into the infectious cDNA backbone of the well established yellow fever vaccine virus strain 17D. This was started at Washington and St Louis University Medical schools. These yellow fever chimeras are further developed commercially by Acambis, Inc. [Rice *et al.* 1989; Guirakhoo *et al.* 2000, 2004] and licensed for manufacture to Sanofi Pasteur. Vero cells serve as the substrate for vaccine virus production. The results from Sanofi Pasteur's lead candidate dengue vaccine phase IIb shows for the first time that a safe and efficacious vaccine against dengue is possible. This candidate vaccine was immunogenic for all four serotypes and may have protected against dengue virus serotype 1, 3, and 4 of the four serotypes [Sabchareon *et al.* 2012]. The candidate vaccine has now progressed to phase III efficacy studies. In the absence of a correlate of protection, large efficacy trials are required to prove efficacy against dengue in the field and to build a robust safety database [Wallace *et al.* 2013].

Live attenuated and live chimeric dengue vaccines, when inoculated into dengue-immune children or adults, have not resulted in enhanced disease caused by vaccine virus [Guy *et al.* 2011]. Risk assessments of vector transmission by vaccine recipients have also been performed by

numerous dengue vaccine developers. The results of published studies indicate a very low likelihood that a vaccine could transmit vaccine-derived dengue viruses to a mosquito [Sardelis *et al.* 2000; Johnson *et al.* 2002, 2004; Higgs *et al.* 2006; McGee *et al.* 2008].

Molecular clone-based strategies for a tetravalent dengue vaccine offer important advantages over traditional attenuation in cell culture. These include a reduced risk of adventitious agents that will also reduce product quality assurance costs and a molecular explanation for attenuation.

Interference observed when mixtures of four dengue viruses are inoculated in susceptible human volunteers must also be studied in genetically modified vaccine viruses [Vaughn *et al.* 2008].

Inactivated virus

Inactivated whole virus vaccines have two advantages since they cannot revert to a more pathogenic phenotype and they are unlikely to interfere with each other in combination. Moreover, induction of cell-mediated and humoral immune responses has been demonstrated with inactivated flavivirus vaccines [Aihara *et al.* 2000].

However, inactivated vaccines express only the part of the viral genome that encodes structural proteins. In the context of dengue immunity and immunopathology, raising antibodies that may not be fully protective may lead to breakthrough infections or enhance infections with wild-type dengue viruses and a requirement of adjuvants for enhancing immunogenicity. Two adjuvants AS03 and AS04 have been incorporated into vaccines licensed for human use and are being explored by the WRAIR/GSK/Oswald Cruz Foundation Killed dengue Vaccine Initiative [Garcon *et al.* 2011; Morel *et al.* 2011].

Subunit vaccines

Recombination subunit approaches offer advantages, including anticipated minimal reactogenicity and freedom from adventitious agents. However, incomplete post-translational processing of proteins can lead to proteins that differ from native proteins and antibody responses [Smucny *et al.* 1995]. Production in mammalian cells may reduce some of these concerns [Konishi and Fujii, 2002]. A phase I study to assess the safety and tolerability of a DEN-1 candidate in healthy US adults was completed and publication is pending results [National Institutes of Health, 2013].

DNA vaccines

Dengue DNA vaccines offer a possible method to raise protective immunity, bypassing the problem of interference seen with multivalent live virus vaccines. DNA vaccines are composed of a plasmid or plasmids containing dengue genes. Tetravalent DNA vaccine inoculated in mice and monkeys successfully raised neutralization antibodies. Monkeys resisted challenge with DEN-1 but not DEN-2 [Whalen, 1996; Apt *et al.* 2006; Raviprakash *et al.* 2006].

A DENV-1 DNA vaccine was evaluated in flavivirus-negative volunteers with the three-dose series at day 0, and at 1 and 5 months. None of the volunteers receiving a low dosage and half of those receiving a high dosage developed neutralizing antibodies [Beckett *et al.* 2011]. More recently, protection has been achieved in a rhesus monkey model by boosting tetravalent DNA vaccination with a tetravalent live attenuated dengue vaccine [Simmons *et al.* 2010].

The DNA approach also carries unique risk which includes the theoretical risk of nucleic acid integration into the host's chromosomal DNA to potentially inactivate tumor suppressor genes or activate oncogenes [Klinman *et al.* 1997; Jechlinger, 2006]. This risk appears to be well below the spontaneous mutation frequency for mammalian cells [Nichols *et al.* 1995; Martin *et al.* 1999]. However, if a mutation due to DNA integration is a part of a multiple hit phenomenon leading to carcinogenesis, it could take many years before this problem becomes evident. Another concern is that foreign DNA might induce anti-DNA antibodies, leading to autoimmune diseases such as systemic lupus erythematosus. However, studies on lupus-prone mice, normal mice, rabbits, and people to date have not validated this concern [Mor *et al.* 1997, Parker *et al.* 1999].

Vectored vaccines

Recombinant poxviruses and adenoviruses expressing foreign proteins have been demonstrated to induce strong humoral and cellular responses in humans against various pathogens. Several live virus vectors such as adenovirus,

alphavirus, and vaccinia virus are designed for direct administration to the host and have been engineered to express DENV E protein for further evaluation as dengue vaccine candidates [Halstead and Thomas, 2013; Wan *et al.* 2013].

Moreover, recombination E proteins expressed from yeast and insect cells and virus-like-particle-based dengue vaccine have been other approaches and under preclinical studies [Arora *et al.* 2013; Mani *et al.* 2013; Wan *et al.* 2013].

Future directions in dengue vaccines

Although no licensed dengue vaccine is available, the increasing knowledge of dengue vaccine development is providing more insights into improved vaccine design.

Several promising dengue vaccine candidates are in preclinical and clinical development, and one has moved into phase III testing. More research is needed on correlation of protection to DENV-2 in the Sanofi Pasteur phase IIb trial despite balanced neutralizing antibody response to vaccination. Economic forces and technologic advances should soon bring one or more dengue vaccines to the market. It remains for the vaccine community to develop and implement plans for the strategic use of dengue vaccines by developing evidence-based policies to target high-risk groups and decrease virus transmission [Wan *et al.* 2013].

There is need to ensure that adequate surveillance is in place and is maintained to detect safety and effectiveness of dengue vaccine during the post-licensure period. Early preparation and understanding of the true burden of disease will be essential for successful vaccine introduction [World Health Organization, 2011] and, with this in mind, the ASEAN Member States Dengue Vaccination Advocacy Steering Committee (ADVASC) convened a regional workshop to review the current status of dengue surveillance and diagnostics in the ASEAN region.

ADVASC recommended an evidence-based approach to strengthening and harmonizing key attributes of dengue surveillance, including case classification, data collection, data analysis, and laboratory testing. Strengthening vaccination policy will require further investment in existing health systems, and recommendations for research and advocacy are also emphasized [Thisyakorn, 2012; Thisyakorn *et al.* 2013].

Conclusion

Dengue virus is the causative agent of a wide spectrum of clinical manifestations, ranging from mild acute febrile illness to classical DF and DHF. DHF is caused by the potentially fatal forms of dengue virus infection, which has become an intractable global health problem.

Vector control has achieved only limited success in reducing the transmission of dengue and there are currently no licensed antivirals to treat dengue. The most effective way to control dengue diseases in the future will include the use of a safe and effective vaccine. Dengue is a unique and complex disease; developing a dengue vaccine has proven equally complex. Although no licensed dengue vaccine is yet available, several vaccine candidates are under development, including live attenuated virus vaccines, live chimeric virus vaccines, inactivated virus vaccines, and live recombinant, DNA and subunit vaccines. The live chimeric virus vaccine is undergoing a phase III clinical trial. Other vaccine candidates have been evaluated in preclinical animal models or are being prepared for clinical trials.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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