Next generation dengue vaccines: A review of candidates in preclinical development

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ABSTRACT

Dengue represents a major public health problem of growing global importance. In the absence of specific dengue therapeutics, strategies for disease control have increasingly focused on the development of dengue vaccines. While a licensed dengue vaccine is not yet available, several vaccine candidates are currently being evaluated in clinical trials and are described in detail in accompanying articles. In addition, there are a large variety of candidates in preclinical development, which are based on diverse technologies, ensuring a continued influx of innovation into the development pipeline. Potentially, some of the current preclinical candidates may become next generation dengue vaccines with superior product profiles. This review provides an overview of the various technological approaches to dengue vaccine development and specifically focuses on candidates in preclinical development.

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1. Introduction

Dengue is a mosquito-borne flavivirus disease that has spread to most tropical and many subtropical areas and creates a significant burden of disease and economic costs in endemic countries [1–4]. As specific dengue therapeutics are not available and disease prevention is limited to vector control measures, the development of a dengue vaccine would represent a major advance in the control of the disease (reviewed in [5]). While no licensed dengue vaccine currently exists, vaccine development has progressed considerably in recent years. Several candidates are currently undergoing either clinical evaluation or preclinical development. These have been reviewed recently [6–9].

This review focuses on dengue vaccine candidates in preclinical development. It is based on published and unpublished data presented by vaccine developers and researchers at the WHO/IVI Informal Consultation on Next Generation Dengue Vaccines and Diagnostics (1–2 November 2010, Atlanta). Additional preclinical candidates from the published literature are included in the review to illustrate the wide range of strategies applied to dengue vaccine development. The first part of the review presents an overview of dengue immunity, challenges to vaccine development and the various technologies used, while the second part provides a more detailed description of specific vaccine projects in preclinical development.

2. Overview of dengue vaccine development

2.1. 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. Multiple DENV can co-circulate in endemic areas (reviewed in [10]). Infection by one serotype confers lasting protection against re-infection by the same serotype, but only transient protection against secondary infection by one of the three heterologous serotypes (reviewed in [9]). Dengue vaccine development efforts therefore aim for a tetravalent vaccine which simultaneously provides long-term protection against all DENV serotypes.

The mechanism of protective immunity against dengue is not fully understood. There is considerable evidence for a major role of antibody-mediated DENV neutralization in protection against infection and disease [9]. However, it is unclear what quantity of neutralizing antibody is needed for protective immunity, and it is increasingly likely that the contribution of neutralizing antibodies to protection will not become known until efficacy trials of candidate vaccines have been completed. In addition, contributions of other immune mechanisms such as cytotoxic T cell responses are
less clear [reviewed in [9,11,12]]. Further research is needed to better define and validate immune correlates of protection for vaccine development [11].

Another challenge for vaccine development is the potentially detrimental role of immune enhancement in dengue pathogenesis [5]. Severe disease is most commonly observed in secondary, heterologous DENV infections. Antibody-dependent enhancement (ADE) of infection has been proposed as the primary mechanism of dengue immunopathogenesis [13,14]. In vitro studies suggest that the capacity of DENV antibodies to contribute to neutralization or enhancement of infection is determined by multiple factors, including antibody specificity, antibody affinity, antibody titre and epitope accessibility [reviewed in [15,16]].

The potential risk of immune enhancement of infection and disease underscores the importance of developing dengue vaccines which produce balanced, long-lasting immunity to all four DENV serotypes. A tetravalent vaccine eliciting protective, neutralizing antibody responses against all serotypes should address theoretical concerns about vaccine-induced ADE. However, vaccine-induced ADE might again become problematic as antibody titres wane post-vaccination.

DENV virions are composed of a lipid envelope modified by the insertion of envelope (E) proteins and premembrane/membrane (prM/M) proteins (depending on the maturation state [17]), surrounding a nucleocapsid composed of capsid (C) proteins and the viral RNA genome [reviewed in [18]]. Human antibodies raised against the DENV virion are mostly targeted at the E and prM proteins [19]. Research to determine the most suitable target epitopes for vaccines is ongoing, and while considerable progress has been made in the characterization of the humoral immune response to DENV, important knowledge gaps still exist. There is evidence suggesting that anti-E antibodies have higher type-specific neutralization capacity and lower ADE potential than anti-prM antibodies. Moreover, most potent DENV neutralizing antibodies identified so far recognize epitopes in domain III of the E protein (EDIII) which is involved in binding of DENV to cell receptors [19–27]. The conundrum of the human antibody response to DENV infection is that most antibodies are specific for domain II of the E protein (EDII) [19], which contains a variety of serotype and flavivirus cross-reactive epitopes. The contribution of the human antibody bias to EDII to sensitization for ADE has not been determined. Because of its antigenic nature, EDIII is considered an antigenic target of particular relevance for dengue vaccine development. New vaccine strategies may have to be developed to enhance the human anti-EDIII response. Antibodies directed against the C protein and against non-structural proteins, such as NS1, have also been detected in DENV-infected individuals, but their role in dengue immunity or immunopathogenesis remains unclear [9].

Dengue vaccine development has been hampered by the lack of an adequate animal model for the disease [28]. Normal mice do not display significant viremia or disease when infected with human DENV isolates. Intracerebral challenge of mice with certain mouse-adapted DENV strains produces a paralysis phenotype, and this intracerebral challenge model has been used to evaluate protective efficacy of vaccine candidates in mice. Furthermore, immunocompromised mouse models have been developed which show some of the characteristics of human disease and can also be used as an in vivo system for studying ADE [29–31]. However, concerns remain about whether the full spectrum of dengue immunity and disease can be modeled in this immunocompromised setting. Humanized mice that lack a murine antibody response but demonstrate some symptoms that re-capitulate human infection are also being investigated [32,33]. Non-human primates (NHPs) are susceptible to infection by DENV and develop viremia, but do not show significant clinical signs of infection. Studies to address protective efficacy of vaccine candidates in NHPs therefore measure prevention or reduction of viremia upon viral challenge [28].

2.2. Technological approaches to dengue vaccine development

A wide range of vaccine technologies has been applied to dengue vaccine development, including live attenuated virus (LAV), purified inactivated virus (PIV), recombinant subunits, virus-like particles (VLPs), and plasmid or viral vectors. Each of these approaches has its advantages and challenges.

LAV vaccines have been shown to produce robust, lasting and broad immunity, including humoral and cellular immune responses [34]. In addition, their production cost tends be lower than for many other vaccine technologies. However, it has often proven difficult to achieve a level of attenuation which optimally balances low reactogenicity (e.g. avoidance of clinical dengue symptoms) with sufficiently high immunogenicity. In addition, LAV vaccines should replicate well in cell culture in order to facilitate their production, while their transmissibility by mosquitoes should be prevented or strongly reduced. Attenuation strategies for live dengue vaccines include serial cell passage (e.g. in Primary Dog Kidney cells). The first tetravalent dengue vaccine was produced in this way [35]. Additionally, introduction of selected mutations or creation of chimeric viruses by recombinant DNA technology can be used. Success of these strategies is however often limited by lack of a detailed understanding of the molecular determinants of virulence due to unavailability of animal models, as described above. There are also concerns about the genetic stability of LAV vaccines, including the possibility of reversion to a more virulent phenotype and the theoretical risk of recombination between vaccine and wild-type viruses. Furthermore, the need to achieve a balanced immune response against all four DENV serotypes presents a particular challenge for multi-component LAV vaccines. Replication interference between the different serotypes of DENV in tetravalent formulations has been observed, resulting in a reduction of neutralizing antibodies to some serotypes, when compared to monovalent formulations. LAV vaccines represent the majority of dengue vaccine candidates in clinical evaluation [36–47], and additional candidates are in preclinical development [48–53].

Nonliving vaccines can have some advantages over LAV vaccines, including reduced potential for reactogenicity and better suitability for immunocompromised individuals. Moreover, achievement of a balanced immune response is facilitated by lack of replication competition between tetravalent vaccine components. However, immune responses to nonliving vaccines tend to be less broad, potent and durable compared to LAV vaccines. Adjuvants have been shown to improve immunogenicity of nonliving vaccines, but safety concerns raised in relation to some adjuvants need to be addressed [54]. Also, access to adjuvants has proven difficult for many vaccine developers.

PIV dengue vaccines are killed wild type virions, containing all DENV structural proteins and viral RNA. This permits induction of an immune response to the three DENV structural proteins. PIV dengue vaccines have not yet reached clinical evaluation, but several candidates are in preclinical development [55,56].

Other nonliving vaccines express particular DENV antigens, which allows for targeting of the immune response to antigens with particular desirable properties (e.g. induction of potent neutralizing antibodies). Recombinant subunit dengue vaccines have been produced in a variety of protein expression systems, including bacterial, yeast, insect and mammalian cells. Most dengue subunit vaccines express truncated versions of the E protein that have deleted transmembrane domains, or simply express EDIII alone. While subunit vaccines often show low reactogenicity, achievement of sufficient immunogenicity usually requires the use of adjuvants. Various recombinant dengue subunit vaccines are in
preclinical development [57–64], and the most advanced candidate [65] is being evaluated in phase 1 clinical trials.

VLP vaccines are virus-like particles that do not contain replicative genetic material, but permit presentation of antigens in a repetitive, ordered array similar to the virion structure, which is thought to increase immunogenicity [66]. Several dengue VLP vaccine candidates are currently in preclinical development [67–70].

DNA vaccines and virus-vectored vaccines allow for in vivo expression of antigens, often in the form of DENV VLPs. One of the advantages of DNA vaccines is their thermostability, which avoids cold-chain requirements for vaccine storage and transport. However, DNA vaccines often face challenges in terms of adequate cellular uptake and expression. Improved DNA vaccine delivery systems have shown some success in addressing these issues [71]. Various DNA dengue vaccines are currently in preclinical development [72–81]. The most advanced DNA dengue vaccine candidate has progressed to phase 1 clinical trials [82].

Virus-vectored dengue vaccines express DENV antigens from a viral vaccine vector. Viral vectors allow for efficient infection of target cells. Moreover, several well-characterized vaccine vector platform technologies exist, some of which are used for licensed vaccines with a well-known safety record [83]. However, there are concerns that pre-existing immunity to viral vaccine vectors could reduce the effectiveness of some virus-vectored dengue vaccines. Several virus-vectored dengue vaccine candidates are currently in preclinical development, including candidates based on non-replicating vectors [84,85], single-cycle vectors [86,87] and replication-competent vectors [88].

Heterologous prime-boost approaches, which use different vaccine types for the priming and boosting steps of an immunization schedule, may allow for optimization of immune responses by combining advantages of different vaccine technologies in a unique order of vaccination. However, the increased complexity of vaccination schedules could make heterologous prime-boost approaches more difficult to implement in the context of immunization programmes. Several heterologous prime-boost approaches are currently evaluated in preclinical studies [89,90].

3. Dengue vaccine candidates in preclinical development

3.1. Recombinant subunit vaccines

Several recombinant subunit vaccine candidates have been developed by the Pedro Kouri Tropical Medicine Institute (IPK) in collaboration with the Center for Genetic Engineering and Biotechnology (CIGB) in Cuba (Table 1). One approach is based on fusion of DENV EDIII to the carrier protein p64k of Neisseria meningitidis. The EDIII-p64k fusion protein is expressed in E. coli. Monovalent vaccine candidates for all DENV serotypes were shown to induce neutralizing antibodies and protect against viral challenge in mice. DENV-1 and DENV-2 monovalent candidates have also been evaluated in NHPs. Monkeys were immunized subcutaneously with four doses of the monovalent vaccine (50–100 µg protein per dose, formulated in Freund’s adjuvant). The monovalent vaccine candidates were found to be immunogenic and provided protection against viral challenge [58,91]. Adjuvants suitable for human use are under evaluation, including N. meningitidis serogroup A capsular polysaccharide (CPS-A) adsorbed on aluminium hydroxide [63]. In another approach, a DENV EDIII-capsid fusion protein was expressed in E. coli and mixed in vitro with oligodeoxynucleotides to obtain particulated aggregates. The aggregated DENV-2 EDIII-capsid fusion protein induced both humoral and cellular immune responses in NHPs and provided partial protection against viral challenge [62]. The different subunit vaccine candidates are also being studied in the context of heterologous prime-boost strategies [64,92].

A subunit vaccine candidate developed by VaxInnate is based on fusion of DENV EDIII to a proprietary form of flagellin (STF2D), which serves as an adjuvant. STF2D contains the toll-like receptor 5 (TLR5) activation domain from Salmonella typhimurium flagellin [53]. This approach links adaptive and innate immunity and is believed to mimic natural infection where antigen and TLR ligands are contained in a single pathogen. Physical linkage of TLR ligand to antigen has been shown to be more specific and efficient than co-administration, requiring lower doses of adjuvant. STF2D fusion proteins can be expressed and purified in E. coli. In immunogenicity studies in mice, monovalent EDIII-STF2D fusion proteins were shown to elicit neutralizing DENV antibodies and provide partial protection against viral challenge. Bivalent DENV-1/3 and DENV-2/4 constructs were then generated by fusing the EDIII domains of two different serotypes to separate attachment points on STF2D. Subcutaneous immunization of mice with three doses (3 µg each) of a DENV-1/3-EDIII-STF2D fusion protein resulted in robust and balanced titres of neutralizing antibodies against DENV-1 and DENV-3. It was found that the choice of antigen attachment points (C-terminal fusion versus insertion via replacement of the flagellin D3 domain) affects immunogenicity. Longevity of the immune response is yet to be studied. Additional bivalent constructs are being generated and tested, with the aim of combining two suitable bivalent vaccine candidates to a tetravalent vaccine.

In a development approach by the International Centre for Genetic Engineering and Biotechnology (ICGEB) in India, a tetravalent chimeric EDIII fusion protein was generated, which consists of the EDIII domains of DENV-1, -2, -3, -4 joined by flexible peptide linkers [59]. This single tetravalent approach avoids the complexities related to producing tetravalent mixtures of monovalent vaccine components. The tetravalent chimeric EDIII protein was expressed in Pichia pastoris. Immunization of mice with the tetravalent vaccine candidate adjuvanted with montanide resulted in neutralizing antibody responses against all DENV serotypes.

A different approach to the development of a single tetravalent subunit vaccine was taken at the Taiwanese National Health Research Institutes (NHRI). A consensus EDIII amino acid sequence was derived by sequence analysis of different DENV-1–4 strains, and the consensus EDIII protein was expressed in E. coli. The single tetravalent vaccine candidate adjuvanted with alum induced neutralizing antibody responses against all DENV serotypes in mice [61]. This vaccine candidate was developed further by fusion of the consensus EDIII with a fragment of the Ag473 lipoprotein of N. meningitidis. The consensus EDIII-Ag473 fusion protein was found to induce higher levels of neutralizing antibodies in mice than consensus EDIII formulated in alum [60]. The intranasal administration of the EDIII-Ag473 lipo-immunogen appears to be due to the stimulation of innate immunity through the TLR2 signaling pathway [94].

3.2. DNA vaccines

A tetravalent DNA vaccine candidate developed by Inovio Pharmaceuticals consists of a DNA plasmid vector that expresses a single ORF comprising the EDIII domains of all four DENV serotypes separated by proteolytic cleavage sites [80]. In vivo expression of a single tetravalent EDIII fusion protein, which is subsequently processed into monovalent EDIII proteins by cellular proteases, aims to ensure that the four EDIII domains are presented in equal ratios in order to facilitate a balanced immune response. A synthetic consensus sequence for the EDIII domain of each DENV serotype was derived by sequence comparison of approximately 15 different strains. Sequences were also human codon optimized to improve expression levels. Administration of the vaccine is via an improved
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Abbreviations: CDC: Centers for Disease Control and Prevention, USA; CIGB: Center for Genetic Engineering and Biotechnology, Cuba; FIOCRUZ: Oswaldo Cruz Foundation, Brazil; GSK: GlaxoSmithKline Biologicals; ICGEB: International Centre for Genetic Engineering and Biotechnology, India; IPK: Pedro Kouri Tropical Medicine Institute, Cuba; NHRI: National Health Research Institutes, Taiwan; NMRC: Naval Medical Research Center, USA; NSTDA: National Science and Technology Development Agency, Thailand; UNC: University of North Carolina at Chapel Hill, USA; UTMB: University of Texas Medical Branch, USA; WRAIR: Walter Reed Army Institute of Research, USA.

DNA delivery system based on electroporation-enhanced transfection, suitable for both intradermal and intramuscular routes. Electroporation-based DNA immunization is currently being tested in phase 1 clinical trials for HIV and influenza prevention and in phase 2 clinical trials for hepatitis C, HPV 16/18 and AML/CML cancer therapeutic studies. Immunization of mice with the tetravalent dengue vaccine candidate, using intramuscular electroporation of three doses (10 μg DNA) at biweekly intervals, resulted in neutralizing antibody titres against all four DENV serotypes. Immunization of NHPs was also shown to elicit robust tetravalent antibody responses. Further studies to assess DENV cross-reactive versus serotype-specific antibody responses are in progress.

Another tetravalent DNA vaccine candidate developed at Kobe University is composed of a mixture of four plasmid vectors, each of which expresses the prM and E proteins (prM/E) of one DENV serotype. Two doses of the tetravalent vaccine (100 μg per dose, at three week intervals) were administered to mice using a needle-free jet injector. Mice were protected against viral challenge 3 weeks after the last dose, and persistence of neutralizing antibodies was observed during a 30-week follow-up [95]. Immunogenicity of the tetravalent DNA vaccine in mice was further increased by co-immunization with DENV-2 VLPs generated by co-expression of prM/E in cell culture. Immunogenicity of the DNA vaccine was also found to be improved by co-immunization with an inactivated Japanese encephalitis vaccine [78].

A similar tetravalent DNA vaccine candidate based on prM/E expression has been developed by the U.S. Centers for Disease Control and Prevention (CDC). Transfection of the recombinant plasmid vectors into cultured cells has been shown to result in secretion of prM/E containing VLPs, which have an antigenic structure similar to DENV virions [72,75]. Immunogenicity of a tetravalent mixture of four monovalent DNA vaccines was evaluated in NHPs (two dose schedule one month apart; 500 μg per monovalent component; administration by intramuscular injection). The tetravalent...
vaccine was found to stimulate a balanced immunity that lasted for 10 months and protected from viral challenge. Pre-existing antibodies against DENV or other flaviviruses were shown to improve immunogenicity of the vaccine. Moreover, there is evidence suggesting that the safety and immunogenicity of the vaccine candidates could be further improved by manipulating antigenic determinants of the E protein. In order to reduce the potential for ADE by cross-reactive antibodies, flavivirus group cross-reactive epitopes and DENV serocomplex cross-reactive epitopes in the E protein were mapped with monoclonal antibody panels and mutated to reduce cross-reactivity, resulting in the formation of cross-reactivity reduced VLPs in cell culture [96]. When evaluated in mice, cross-reactivity reduced DENV-1 and DENV-2 VLP vaccines continued to elicit high levels of neutralizing antibodies, but showed reduced ADE potential compared to wild type vaccines both in vitro (K562 cell-based assay) and in vivo (AG129 mouse model [29,97]). Immunogenicity of the cross-reactivity reduced vaccine was further improved by incorporating a CD4 epitope from the transmembrane domain of West Nile virus E protein into the expression construct.

In a single tetravalent approach developed at the U.S. Naval Medical Research Center (NMRC), a prM/E construct containing a chimeric “shuffled” E protein is expressed from a plasmid vector [76]. Various chimeric E proteins containing sequence portions of all DENV serotypes were derived by DNA shuffling technology. The shuffled constructs were screened for antigen expression in vitro and for immunogenicity in mice. Three selected constructs were evaluated as single tetravalent DNA vaccine candidates in NHPs. Two of these candidates were found to induce tetravalent neutralizing antibody responses in the majority of animals and to provide partial protection against viral challenge.

3.3. VLP vaccines

A VLP dengue vaccine candidate developed by Cytos Biotechnologie is based on chemical coupling of recombinant EDIII to carrier VLPs derived from bacteriophage Qβ [98]. A similar approach has recently been applied to the development of a West Nile virus vaccine [99]. About 60–90 EDIII molecules can be coupled to one VLP carrier, resulting in the formation of a highly repetitive, ordered array of antigen, which is expected to increase immunogenicity. Qβ VLPs also contain E. coli-derived ssRNA, which has been shown to be required for IgG isotype switching in mice immunized with Qβ VLPs [100,101] and is used as a built-in adjuvant to improve the antibody response to Qβ VLP-based vaccines. VLPs can be economically produced in E. coli, resulting in 2 g/l culture of GMP grade material. Qβ VLP based vaccine candidates are in advanced clinical evaluation for other target antigens. Four monovalent dengue vaccine candidates were generated by coupling the EDIII domain of the different DENV serotypes to the carrier VLP. Immunogenicity studies in mice were performed to compare monovalent vaccines individually and in a tetravalent formulation (three doses at 10 day intervals; 50 μg per dose of monovalent vaccine and 200 μg per dose of tetravalent vaccine; subcutaneous administration with alum). The neutralizing antibody response observed to DENV-4 was weaker than for the other serotypes. However, an improved formulation with increased representation of the DENV-4 monovalent component induced a potent neutralizing antibody response against all four serotypes.

VLP vaccine candidates have also been developed by the ICGB. In a first approach, DENV E was expressed as a fusion protein with Hepatitis B virus surface antigen (HBsAg) in P. pastoris [67]. HBsAg expressed in P. pastoris has been shown to assemble into highly immunogenic VLPs. Furthermore, high protein yields (up to 7 gram HBsAg per liter culture [102]) make P. pastoris an attractive system for affordable production of protein-based vaccines. While expression of EDIII alone does not result in VLP formation, mosaic VLPs composed of varying ratios of EDIII-HBsAg and HBsAg could be generated by co-expression of the EDIII-HBsAg fusion protein with 1–4 copies of HBsAg. In a second approach, a fusion protein containing the E protein ectodomain (ectoE) and 30 amino residues of the prM protein was expressed in P. pastoris, which also resulted in VLP formation. Structural characterization of the VLPs, their functional evaluation for immunogenicity, and evaluation of prime-boost protocols combining VLP vaccine candidates with rAd5 vectored vaccine candidates are planned.

In a VLP vaccine development approach employed at Kobe University, prM/E is expressed from a plasmid vector transfectated into cell culture-based expression systems and secreted VLPs are purified. A DENV-2 VLP vaccine candidate was found to be immunogenic and protective in mice [103]. Expression of VLPs by transient transfection of insect cells (SF9 cell line) resulted in 10–100-fold improved yields compared to mammalian expression systems [69]. Immunogenicity of the DENV-2 VLP vaccine candidate in mice could be improved by co-immunization with a DNA vaccine expressing prM/E, which also functions as a CpG adjuvant.

3.4. Virus-vectored vaccines

A virus-vectored dengue vaccine developed by ICGEB is based on expression of a tetravalent chimeric EDIII fusion protein from a replication-deficient adenovirus vaccine vector [85]. Advantages of adenovirus vaccine vectors include a large insert capacity, efficient delivery and high expression levels of antigens in a variety of cells, and a well-documented human safety record. Immunization of mice with two doses of the single component tetravalent dengue vaccine candidate (administered 30 days apart by the intraperitoneal route) resulted in balanced neutralizing antibody responses against all four DENV serotypes. Of note, prior immunity to the adenovirus vaccine vector did not impair the potency of the dengue vaccine candidate. Presence of antibodies against the vector in the sera of immunized mice enabled uptake of the vector into U937 human monocytes, which are not susceptible to infection in the absence of antibodies against the vector. This effect is presumably mediated by the Fc receptor pathway [104]. The results suggest that low levels of pre-existing antibodies against the adenovirus vector may in fact even facilitate uptake of the virus-vectored dengue vaccine into antigen-presenting cells.

Another virus-vectored vaccine approach developed by GenPhar in collaboration with the NMRC is based on expression of DENV prM/E from a replication-deficient complex adenovirus vaccine vector capable of accommodating multiple large antigen inserts [84]. The tetravalent vaccine is composed of two bivalent vaccines, each of which expresses prM and E proteins of two different DENV serotypes. NHPs were immunized with two doses of tetravalent vaccine 8 weeks apart, followed by viral challenge at 3–6 months. Neutralizing antibody responses were induced against all serotypes. Viremia from challenge with DENV-1 and DENV-3 was blocked, while viremia from challenge with DENV-2 and DENV-4 was significantly reduced. Further development of the vaccine constructs, including optimization of all prM and E gene sequences for human codon usage, resulted in an improved tetravalent formulation, which induced a more balanced immune response to all four serotypes. The candidate vaccine is scheduled to enter clinical evaluation shortly.

A virus-vectored vaccine candidate developed at the University of North Carolina at Chapel Hill (UNC) is based on expression of DENV antigens from a single-cycle Venezuelan equine encephalitis (VEE) virus vaccine vector [86]. Packaging technologies based on helper RNAs allow for the production of infectious single-cycle VEE virus particles also referred to as virus replicon particles (VRPs). VEE VRPs have been shown to infect human dendritic cells and express
high levels of recombinant antigens, inducing both innate and adaptive immune responses. Different monovalent vaccine candidates expressing DENV prM/E proteins, C-terminally truncated E proteins (E81, E85 [81% or 85% of E]) or EDIII were evaluated for immunogenicity in mice. Vaccine candidates expressing truncated E proteins were found to elicit higher neutralizing antibody titres than candidates expressing prM/E. Immunogenicity and protective efficacy of the monovalent candidates were evaluated further in NHP studies. Three doses (10⁶ IU each) of a monovalent vaccine candidate expressing either DENV-3 E85 or DENV-3 prM/E were administered subcutaneously at weeks 0, 7, and 25, and viral challenge was performed at week 41. Higher neutralizing antibody titres were observed with the DENV-3 E85 based vaccine, which also completely protected all monkeys against viral challenge, while immunization with the DENV-3 prM/E based vaccine resulted in only partial protection. Neutralizing antibodies against DENV-3 E85 were serotype-specific and largely directed against EDIII epitopes. Immunogenicity and protective efficacy studies of a tetravalent E85 based vaccine candidate in NHPs are in progress.

Another approach taken at the University of Texas Medical Branch (UTMB) is based on a single-cycle, capsid-gene deleted West Nile virus vaccine vector [105]. Infectious single-cycle WNV particles are produced by packaging technologies, which supply the capsid protein in trans. Infected target cells are unable to produce viral progeny, but the single replication cycle results in efficient production of antigen including secretion of VLPs from infected cells. A DENV-2 vaccine candidate was generated by replacing the prM/E genes of the WNV vaccine vector with the prM/E genes of DENV-2 [87]. The vaccine was tested for potency and efficacy in a mouse model of dengue pathogenesis (Ag129 mice [97]). A single intraperitoneal immunization of mice produced a dose-dependent DENV-2 neutralizing antibody response and resulted in a protective effect against viral challenge.

A virus-vectored dengue vaccine candidate developed byThemis Bioscience and Institut Pasteur is based on expression of a single tetravalent DENV antigen construct from a live attenuated measles virus vaccine vector [88]. This vaccine vector technology allows for integration of large antigen inserts and has been shown to induce strong neutralizing antibodies and cellular immune responses even in the presence of pre-existing immunity to measles virus. The dengue vaccine candidate expresses a construct containing the EDIII domains of DENV-1-4 as well as the DENV-1 M protein ectodomain (ectoM). The single component tetravalent vaccine-induced neutralizing antibodies against all DENV serotypes in mice. Inclusion of ectoM in the vaccine construct was found to provide an adjuvant activity. Following further improvements of the vaccine construct, immunogenicity and efficacy studies have been initiated in NHPs. Establishment of a GMP process and initiation of clinical trials are planned in 2011 and 2012, respectively.

3.5. Purified inactivated virus vaccines

A purified psoralen-inactivated DENV-1 vaccine candidate has been developed by the NMRC [56]. Following immunogenicity studies in mice, the monovalent candidate adjuvanted in alum was evaluated in NHPs using a three-dose schedule (intradermal injection, biweekly intervals). Immunized monkeys showed a DENV-1 neutralizing antibody response and reduced duration of viremia upon viral challenge on day 132, suggesting that the vaccine candidate provides partial protection in NHPs.

PIV vaccine candidates are also being developed by GlaxoSmithKline (GSK) in collaboration with the Walter Reed Army Institute of Research (WRAIR) and the Oswaldo Cruz Foundation (FIOCRUZ) [55]. Different approaches for virus inactivation and purification and various adjuvant systems are currently under evaluation. A pilot study was carried out in NHPs to test a tetravalent inactivated virus vaccine candidate together with different proprietary adjuvant systems (AS01, AS03, AS04). Two doses of adjuvanted vaccine were given 30 days apart, followed by viral challenge 4 months later. All immunized animals were found to be protected against viral challenge. The highest neutralizing antibody responses were obtained with the AS03 adjuvant system. Clinical trials of the adjuvanted PIV vaccine are planned to start in 2012, with a first assessment of protective efficacy envisaged for 2014–15.

3.6. Live attenuated virus vaccines

A live attenuated dengue vaccine candidate developed by FIOCRUZ uses the live attenuated yellow fever vaccine 17DD strain as a genetic backbone. Chimeric YF 17D/DEN viruses were constructed by replacing the YFV prM/E genes with those of DENV [106]. Monovalent dengue vaccine candidates for different DENV serotypes were generated and evaluated in NHPs. In attenuation studies, the monovalent vaccines produced only low levels of viremia and showed reduced neurotropism compared to the YF 17D vaccine. Neutralizing antibody responses and protection against viral challenge were also shown [48–50]. DEN viruses used for the construction of chimeric YF 17D/DEN viruses in this vaccine project include strains isolated from Latin American patients, while the DENV strains that provided the basis for a tetravalent dengue vaccine candidate developed by Sanofi Pasteur, which is currently in advanced clinical evaluation [44–47,107], were isolated from Asian patients. Different sets of chimeric YF 17D/DEN viruses are thus available for the development of a tetravalent live attenuated dengue vaccine.

Another live attenuated vaccine candidate has been developed in collaboration between Chiang Mai University, Mahidol University, the Thai National Science and Technology Development Agency (NSTDA) and BioNet-Asia [51,52]. DEN/DEN chimeric viruses were constructed which contain the prM/E coding region of recent dengue clinical isolates in the genetic background of attenuated DENV. Monovalent vaccine candidates have been evaluated for neurovirulence and immunogenicity in mice. Safety and efficacy studies in NHPs are planned.

3.7. Heterologous prime-boost approaches

A heterologous prime-boost approach developed by the NMRC uses a DNA vaccine candidate and a virus-vectored vaccine candidate [89]. The monovalent DNA vaccine, which has been evaluated in a phase 1 clinical trial, consists of a plasmid vector expressing DENV-1 prM/E and is administered intramuscularly with a needle-free Biojector system [82]. The virus-vectored vaccine expresses the same DENV-1 prM/E sequence from a single-cycle VEE virus vector. A three-dose prime-boost regimen was evaluated in NHPs, which consists of two doses of the DNA vaccine at days 0 and 28 followed by intramuscular injection of the virus-vectored vaccine at day 117. When compared to homologous three dose schedules of either the DNA or the virus-vectored vaccines, the heterologous prime-boost approach was found to improve neutralizing antibody responses and protection against viral challenge [89].

Another heterologous prime-boost approach developed by the NMRC and the WRAIR uses non-replicating vaccines for priming, followed by boosting with a LAV vaccine candidate [90]. Two non-replicating vaccines were evaluated: a tetravalent DNA vaccine composed of plasmid vectors expressing DENV prM/E [82] and a tetravalent PIV vaccine consisting of formalin-inactivated DENV adjuvanted with alum [55]. The tetravalent LAV vaccine candidate, which was developed by GSK and WRAIR using serum passage of clinical DENV isolates in PDK cells, has reached phase 2 clinical trials [42]. To determine which of the two non-replicating vaccine candi-
dates was more effective for priming, a DNA-DNA-LAV vaccination schedule (at -30, 0 and 60 days) was compared to a PIV-LAV vaccination schedule (at 0 and 60 days) in NHPs. Both regimens were found to elicit neutralizing antibody responses. However, priming with the DNA vaccine provided only partial protection against viral challenge 6 months after the last dose, as evidenced by the observation of breakthrough viremia. By contrast, priming with one dose of PIV vaccine followed by a LAV vaccine booster dose two months later resulted in complete protection against challenge with any of the four DENV serotypes [90].

While heterologous prime-boost strategies may offer advantages over other vaccination approaches, recent investigations with West Nile virus vaccine candidates suggest that the order of antigens used in a heterologous prime-boost approach was important in terms of the specificity and magnitude of the immune response [108]. Similar results can be expected for dengue vaccines.

4. Conclusions

Efforts for dengue vaccine development have faced multiple challenges, including the need to induce a balanced and lasting immunity against four DENV serotypes, uncertainties around immune correlates of protection, potential immune enhancement of disease, and lack of suitable animal model. 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 [44–47] and several other candidates in earlier stages of clinical evaluation [36–43,65,82]. The majority of dengue vaccine candidates currently in clinical development are based on the same immunization regimen (namely a tetravalent LAV) derived using different technologies. However, some uncertainties around the utilization of LAVs remain, including potential imbalances in immunity due to immune interference.

A large number of diverse dengue vaccine candidates are in preclinical development. These preclinical candidates ensure a continued influx of innovation into the vaccine pipeline, which is critical for maximizing the chances of success for dengue vaccine development. One possible scenario is that a first generation of licensed dengue vaccines will arise from candidates currently in clinical development, while some of the current preclinical stage candidates could become next generation dengue vaccines licensed at a later stage. Next generation dengue vaccines could serve to complement existing vaccines. For example, prime-boost approaches combining live and non-replicating vaccines may allow for balancing the relative advantages and shortcomings of these technologies. Furthermore, next generation dengue vaccines could have a superior product profile compared to existing vaccines, including efficacy, dose-scheduling, and stability. There are indications that candidates which are currently in advanced clinical development may require long, multi-dose immunization schedules (e.g. three doses over a 12 month period). Next generation dengue vaccines could have more compressed schedules requiring fewer doses, or characteristics that make them more suitable for certain target groups (e.g. particular age groups, immunocompromised individuals, travelers). Finally, next generation dengue vaccines could generate much-welcomed competition, leading to more affordable and cost-effective vaccines, which would facilitate their use particularly in endemic developing countries.

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