Development of Dengue Vaccines: The Role of Animal Models

Brian J. Morrison1*, Maya Williams1, and Kevin Porter2

1Department of Viral and Rickettsial Diseases, Naval Medical Research Center, USA
2Directorate of Infectious Diseases, Naval Medical Research Center, USA

Abstract

Dengue virus remains the most important arbovirus worldwide, and dengue disease poses a significant public health threat. Infection by one dengue serotype does not produce life-long protection against the other three serotypes, and severe disease may be due to non/poorly-neutralizing antibodies to heterologous or homologous dengue serotypes. As such, development, validation, and implementation of a protective vaccine against all four serotypes of dengue virus are considered to be the best approach for control of dengue disease. The Sanofi Pasteur Dengvaxia® dengue vaccine has been licensed in several endemic countries. However, it is clear that additional vaccine candidates are needed as the Dengvaxia® vaccine has several limitations including: (i) reduced efficacy in flavivirus naïve individuals; (ii) potential safety issues in naïve individuals; and (iii) a delivery regimen of three shots over a 12 month period. The limitation in our current understanding of dengue vaccine efficacy and dengue disease pathogenesis can in part be attributed to the lack of an ideal animal model for testing candidate vaccines. Importantly, improved dengue vaccine development can be addressed by better small animal models for testing dengue vaccine candidates and understanding dengue disease immunopathology. This review will assess the variety of animal models that exist for testing dengue vaccine candidates including, non-human primates, wild type and non-wild type mice, and humanized mice.

INTRODUCTION

Dengue viruses (DENV) are capable of causing severe dengue disease and are the cause of one of the most common vector-borne viral illnesses worldwide. DENV and the insect vectors responsible for their transmission are also spreading globally, including into areas of the U.S. [1,2]. DENV is a single-stranded positive sense RNA virus that belongs to the family Flaviviridae [3] and consists of four distinct serotypes (DENV-1, -2, -3, and -4). The estimated number of global infections per year is 390 million [4], resulting in approximately 500,000 hospitalizations and 20,000 deaths [1]. Infection with DENV is asymptomatic in the majority of cases, but can lead to mild or severe disease, including dengue hemorrhagic fever and dengue shock syndrome. The most effective and cost-efficient method to control dengue is the development of a vaccine that induces protective immunity against all four serotypes of DENV [5,6].

Recently, the Sanofi Pasteur dengue vaccine, Dengvaxia® (formerly known as CYD-TDV), has been licensed in several endemic countries [7-10]. However, recent results have made it clear that further dengue vaccines are needed. These results for the Dengvaxia® vaccine include: (i) reduced efficacy in flavivirus naïve individuals [7,10], in one study 43.2% efficacy was seen for seronegative status individuals compared to 83.7% for seropositive status individuals at baseline [10]; (ii) potential safety issues in naïve individuals (for a description of safety concerns please refer to [11]); and (iii) a delivery regimen of three shots over a 12 month period [7,10]. Various additional dengue vaccine approaches have been tested including, amongst others, adjuvanted, tetravalent purified inactivated virus (PIV)[12], additional tetravalent live attenuated viruses (LAVs), including vaccines from the National Institute of Allergy and Infectious Diseases[13-17], and Takeda Pharmaceutical Company[18-20] that are entering phase III testing, as well as
Recently, there has been interest in developing dengue live virus human challenge models in order to assess the potential efficacy of vaccine candidates in fewer subjects prior to testing in endemic areas with a larger number of subjects[22,23]. Although a human challenge model would provide insight into immune responses to dengue virus [24], help to define correlates/surrogates of protection [25], and could greatly accelerate vaccine development, safety remains a consideration [26,27]. Unlike similar human challenge models for malaria, influenza, and enteric bacterial infections, there is no currently available licensed specific therapy for dengue virus. Therefore animal models remain critical for understanding dengue disease pathogenesis and for preclinical testing of dengue vaccines.

Host immune responses to natural infection and dengue vaccination are incompletely understood. Clinical trials with the Sanofi Pasteur dengue vaccine demonstrated that neutralizing antibodies alone are not predictive of protection [9] and correlates of protection need to be further assessed. Additionally, dengue disease pathogenesis is complex, believed to be influenced by multiple factors and remains incompletely understood [28,29]. The limitation in our current understanding of dengue vaccine efficacy and dengue disease pathogenesis can, in part, be attributed to the lack of an ideal animal model [30]. Current animal models include non-human primates (NHP), wild type (WT) and non-wild type mice, and humanized mice, and each has advantages and limitations (Table 1). This review will provide an update on the field with a focus on the need for improved animal models for dengue vaccine testing that strike a balance between susceptibility to infection and development of relevant humanized immunity (for previous reviews of the field please refer to [30-34]). An improved animal model would be extremely useful for vaccine development by allowing for relevant exploration of: (i) candidate dengue vaccine protection; (ii) correlates of protective immunity; and (iii) dengue disease pathogenesis.

**Non-Human Primates**

Pre-clinical testing of candidate vaccines has demonstrated the utility of NHP for testing, as well as some of the disadvantages [12,35-45]. NHP are currently utilized for testing the safety and protection of dengue vaccine candidates before they can be advanced to human trials, with several dengue candidate vaccines in clinical trials having previously shown efficacy in NHP [46]. NHP studies utilizing the dengue 1 monovalent DNA vaccine (D1ME100) formed the basis for selection of the first dengue DNA vaccine for evaluation in humans [35,36,47]. These studies demonstrated production of neutralizing antibodies following vaccination, protection or reduction in viremia, and proof of concept that dengue DNA vaccines can protect NHP from challenge with wild type virus. Additional NHP studies have demonstrated immunogenicity and protective effect of single and tetravalent chimeric yellow fever dengue vaccines [39-41], immunogenicity for LAV vaccines [45] including induction of neutralizing antibodies against all four serotypes of DENV [37], and immunogenicity and protective effect of tetravalent formulations of chimeric dengue (DENVax) [19, 42].

NHP studies have the advantage of allowing vaccines to be evaluated for immunogenicity, including neutralizing antibody response and cell mediated immunity, and for immunization schedules that are more aligned to those of humans. Several NHP species can sustain a period of measurable viremia following DENV infection [48]. Viremia in NHP is useful as after DENV challenge vaccine protection can be tested by determining its ability to reduce or eliminate measurable viremia after the animal is inoculated with a specific DENV serotype. Additional NHP studies have demonstrated proof of concept that dengue DNA vaccines can protect NHP following vaccination, protection or reduction in viremia, and studies demonstrated production of neutralizing antibodies for evaluation in humans [35,36,47]. These NHP studies utilized the dengue 1 monovalent DNA vaccine (D1ME100) formed the basis for selection of the first dengue DNA vaccine for evaluation in humans [35,36,47]. These NHP studies have the advantage of allowing vaccines to be evaluated for immunogenicity, including neutralizing antibody response and cell mediated immunity, and for immunization schedules that are more aligned to those of humans. Several NHP species can sustain a period of measurable viremia following DENV infection [48]. Viremia in NHP is useful as after DENV challenge vaccine protection can be tested by determining its ability to reduce or eliminate measurable viremia after the animal is inoculated with a specific DENV serotype. However, NHP do not develop clinical manifestations of dengue fever, and thus, the utility of this preclinical model in studying dengue disease pathogenesis is limited (reduction in viremia and antibody

<table>
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<tr>
<th>Model</th>
<th>Advantages</th>
<th>Limitations</th>
<th>References</th>
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<tbody>
<tr>
<td>Non-Human Primate</td>
<td>*Can sustain viremia for a measureable period</td>
<td>*Costly</td>
<td>[12,19,35-45,47]</td>
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<td></td>
<td>*Immunogenicity (neutralizing antibody response)</td>
<td>*Lack of clinical manifestation of disease</td>
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<td>*Immunoization schedules that are more aligned to humans</td>
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<td></td>
<td>*Useful for vaccine development studies</td>
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<td>Wild Type Mice</td>
<td>*Cheap, reproducible, large numbers can be utilized</td>
<td>*Lack of clinical manifestations</td>
<td>[19,38,50-60]</td>
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<td></td>
<td>*Induction of mouse immune response</td>
<td>*Does not mimic infection route</td>
<td></td>
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<td></td>
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<td>*Does not sustain infection</td>
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<td>AG129 Mice</td>
<td>*Cheap, reproducible, large numbers can be utilized</td>
<td>*Lack of competent IFN pathway leading to poor immune responses</td>
<td>[18,45, 60-65, 68-74]</td>
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<td></td>
<td>*Useful for antiviral studies</td>
<td>*Few viral strains useful</td>
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<td></td>
<td>*Sustain viremia</td>
<td>*Few clinical manifestations</td>
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<td>IFNAR− Mice (C57BL/6)</td>
<td>*Cheap, reproducible, large numbers can be utilized</td>
<td>*Lack of competent IFN type I pathway</td>
<td>[66,67]</td>
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<td></td>
<td>*Sustain viremia</td>
<td>*Few viral strains useful</td>
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<td>*CD8+ T cell response</td>
<td>*Few clinical manifestations</td>
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<td>Humanized Mice</td>
<td>*Clinical manifestations</td>
<td>*No/poor induction of human anti-DENV IgG</td>
<td>[75-82]</td>
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<td>*Induction of human cytokines/chemokines and cellular immune response</td>
<td>*Costly and time consuming to prepare</td>
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<td></td>
<td>*Induction of human neutralizing Anti-DENV IgM</td>
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Wild Type and Interferon Receptor-Deficient Mice

Compared with NHP, mouse models are less expensive and can be manipulated more readily. A WT mouse model, an AG129 mouse model, in which mice are deficient in interferon (IFN) α/β and γ receptors, and an IFNAR-/- mouse model, lacking the type I IFN receptor, have been widely used to explore DENV infection [31,49]. However, these models possess shortcomings for vaccine studies. Several WT mouse dengue vaccine studies utilize lethal DENV challenge to assess protective efficacy of candidate vaccines [19,38,50-54]. The WT mouse model has limited utility in vaccine research because it does not mimic the natural route of infection, only exhibits a rare clinical manifestation of dengue disease (encephalitis) observed in humans and cannot sustain DENV measurable viremia from any of the serotypes [52,55]. However, immune responses, including induction of CD4 and CD8 T cell responses, have been reported for DENV vaccine candidates tested in WT mice protected from lethal dengue encephalitis, informing on the role of antiviral T cells in vaccine response [52]. Induction of protective neutralizing antibody responses have also been elicited in WT mice following vaccination with DENV envelope protein subunit vaccines [38,56,57], or after combination vaccines composed of DNA priming and protein boosting with either envelope or non-structural proteins [58,59]. Importantly, vaccine protection against viral challenge demonstrated in WT mice have been subsequently demonstrated in NHP models [38], supporting the use of WT mice for assessing vaccine candidates. Adoptive transfer studies, utilizing the immunogenic response WT mice produce (serum antibodies, T, and B cells) transferred into naïve AG129 prior to virus challenge is one way to overcome some of the limitations of the WT model [60]. The AG129 mouse model has found some utility in vaccine studies as the AG129 mice do demonstrate disease pathology and viral replication following DENV injection.

The AG129 mouse model has been used for studying DENV vaccine candidates’ protective efficacy with protective effects assessed including increased mean survival time after lethal virus challenge, reduced viremia and pathology, and induction of immunogenicity including neutralizing antibodies [18,45,61,62]. The AG129 mouse model can be of use for exploring the humoral arm of the adaptive immune system in isolation, but the lack of IFN α/β and γ receptors decouples the model’s cellular immune response and essentially renders the model’s T cell responses ineffective. The lack of a competent IFN pathway will impact the functionality of the antibody responses. These features limit the AG129 model’s utility for evaluating the interaction between the humoral and cell-mediated immune responses in vivo. Moreover, the wild type mouse and AG129 mouse models have mouse immune systems and do not take into account potential differences with the human immune system. Furthermore, the AG129 model demonstrates heterogeneity in the susceptibility of mice to various strains of dengue virus [63], and demonstrates few clinical manifestations of dengue disease [64]. Recently, a non-mouse-adapted DENV-2 strain has been used in the AG129 model that does mimic some of the aspects of dengue pathogenesis [65]. However, this is for a single strain of DENV, and the relevance to human infection is not definitive.

An additional mouse model, IFNAR-/- mice, has some advantages over the AG129 model for investigating immune responses and vaccine testing. IFNAR-/- mice have been used to demonstrate the protective role of DENV-specific CD8 T cells [66]. Additionally this study identified DENV-specific CD8 T cell epitopes, and peptide vaccination with these epitopes resulted in enhanced control of DENV infection and viral load. IFNAR-/- Human Leukocyte Antigen (HLA) transgenic mice have also been used to assess T cell responses restricted by human HLA molecules. Several new human T cell epitopes from nine out of the ten DENP proteins have been identified using these mice [67]. This model has the advantage of better modeling the T cell response patterns observed in humans and allows the investigation of T cell responses relevant to dengue virus vaccine design, including vaccines that may be dependent on the IFN-γ pathway. While the IFNAR-/- mouse and AG129 mouse models can be invaluable tools for evaluating therapeutic drug candidates [68-72], or other therapies [73,74], to treat DENV disease, they are not sufficient to improve our current understanding of human immune responses induced by experimental dengue vaccines. Thus, the development of humanized mouse models able to generate a surrogate human immune system is a highly pursued goal for investigating candidate vaccines prior to clinical trial.

Humanized Mice

Humanized mouse models, defined as immunocompromised mice infused with human CD34+ hematopoietic stem cells (HSCs), have been developed, but have been shown to be suboptimal for reconstitution of the human immune system [75]. Immunodeficient humanized mouse models, including NOD/SCID and NOD-SCIDIL2Rγnull, have been used for dengue virus infection/pathogenesis research, and infected mice present with some clinical manifestation of disease as in humans such as viremia, fever, rash, thrombocytopenia, and erythema [76-78]. Production of human specific anti-DENV IgM and human cytokines have been detected following infection [79-80], and infected human cells have been detected in bone, blood marrow, and spleen cells [76]. In one study, humanized NOD-SCIDIL2Rγnull were used to assess infection with different DENV-2 strains [77]. In this model, clinical signs of dengue fever were noted according to the infecting virus genotype, but anti-dengue antibodies were only detected in mice receiving the most virulent virus. One rationale for why this is the case is the lack of human immune factors (interleukins) that are necessary to support B cells. In a subsequent study, NSG mice (NOD.Cg-Prkdcscid IL2rgtm1Wjl/szJ) reconstituted with human cord blood and injected intradrernally with DENV-2 demonstrated signs of dengue disease, human lymphocyte permissivity to infection by a DENV-2 strain and subsequent production of various human cytokines and chemokines [80]. Although cellular immune responses occur following infection, isotype class switching (from IgM to IgG) of antibodies does not typically occur [81].

Isotype class switching limitations might be partially explained by the lack of expression of HLA molecules in the
mouse lymphoid system, resulting in suboptimal human T cell reconstitution and limited immunoglobulin class switching by B cells. While these immunocompromised mouse models have allowed partial reconstitution of human B and myeloid cell lineages, they have been unsuccessful in reconstituting a functional T cell lineage. To improve on this, studies have used immunocompromised mice engrafted with human HSCs and co-transplanted with human fetal thymus and liver tissue implanted under the kidney capsule (BLT mice) [79,82]. Implantation of the human fetal thymus and liver tissue provides a microenvironment for education of human T cells on autologous tissue. BLT-NSG mice have demonstrated enhanced titers of neutralizing IgM antibody compared to stem cell-only engrafted mice, and these mice also generated an HLA-A2 restricted dengue virus-specific T cell IFN-γ response following peptide stimulation [79]. DENV infection of BLT mice have also been shown to result in sustained viral infection, the induction of human cytokines/chemokines, induction of anti-dengue IgM (but not IgG response), induction of additional DENV-specific T cell responses, and have shown utility as a model for assessing antiviral therapy testing [82]. BLT mice represent an improvement as a mouse model of dengue infection and immune response compared to immunocompromised/humanized only mice. However, generation of BLT mice is time-consuming, and xenotransplant of tissue to foster T cell development/functionality is not as robust as a transgenic model where human HLA is present constitutively and from birth. Recently, such a model has been developed, humanized HLA-DR4.RagKO.II.L2RgammacOKO.NOD (so called DRAG) mice that express human HLA-DR4 and develop a more functional human T and B cells (capable of producing specific human IgG) [83,84]. These results suggest that humanized mice models may soon find use in assessing immunogenicity and protective efficacy of dengue vaccine candidates.

CONCLUSION

Current animal models for studying dengue candidate vaccines each have strengths and weaknesses, and care must be taken when comparing or extrapolating animal results to those in humans. However, animal models have found utility for testing safety and protective efficacy of dengue vaccines and antiviral therapies. They have also offered insight into dengue infection immunology and immunopathology. In part due to the lack of an ideal, existing, small animal model of dengue disease, human infection models for dengue virus are being developed. Live virus human challenge models, where attenuated viruses are intended to produce a mild, uncomplicated dengue illness, may provide insight into immune correlates of protection and will benefit the development of dengue vaccines. Correlation of results obtained from a human live virus challenge model to an ideal humanized mouse model for dengue would further allow a platform for conducting hypotheses testing from results obtained in human studies. Improvements on humanized mouse models may offer additional insights into dengue immunopathology following infection and may improve our current understanding of human immunoprotective responses to vaccines. Utilization of an improved humanized mouse model for DENV vaccination and infection challenge would: (i) allow assessment of a variety of DENV serotypes, strains, and vaccine candidates; (ii) allow assessment of clinical manifestations of disease and potential assessment of severe disease; and (iii) provide an agile platform to refine variables for future use in human vaccine clinical trials.

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