

Review on murine models available for dengue vaccine development

Akash Parthi Bendran¹, Vikneswaran Murugaiyah² and Surash Ramanathan^{1*}

¹Centre for Drug Research, ²School of Pharmaceutical Sciences,
Universiti Sains Malaysia, 11800 Penang, Malaysia

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Dengue is one of the most lethal and wide spread diseases with an estimated 5,00,000 cases being reported each year. Despite this, there are no targeted antivirals or vaccines approved for treatment. This could be attributed to the lack of appropriate animal models available to carry out dengue vaccine testing. A developed rodent model will be ideal for vaccine testing in laboratory. One major setback is that wild type mice are resistant to dengue virus replication. Interferon deficient mice are considered to be the most accurate model for vaccine testing as it recapitulates all the vital characteristics of severe dengue disease manifestation. Therefore, for development of dengue vaccine, interferon deficient mouse model will be a rigid model for testing and development of dengue-specific vaccines or antivirals.

Keywords: Animal models, Antiviral drugs, Dengue virus, Immunopathogenesis, Pathogenesis, Vaccines.

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Introduction

Dengue is a mosquito-borne viral infectious disease caused by four distinct virus serotypes (DENV1-4) belonging to the family Flaviviridae. Dengue is wide spread in the Asia, Pacific, Caribbean, America, and Africa. Infection to humans is transmitted through the bites of *Aedes* mosquitoes. Dengue fever is one of the most prevalent vector-borne diseases in the world; an estimated 5,00,000 cases of severe dengue require hospitalization each year, of which a very large proportion is in children. Fatal cases were about 2.5 %, fatalities could ascend to twice the figure¹⁻². Recently, Malaysia experienced an increase in new dengue cases. The cumulative number of reported cases and deaths were 53, 246, and 147, respectively³.

The four distinct serotypes are all causative agents of dengue fever (DF), dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF). The four serotypes are antigenically distinct, showing 65-70 % sequence homology⁴. Infection with one serotype gives minimum protection against the other three serotypes. This minimum protection against the other three serotypes lasts for six months only, after which the individual is susceptible to sequential infection with the other three serotypes. An infection with one serotype

gives lifelong protection against reinfection with the same serotype, homologous serotype⁵. Primary infection does not manifest into life threatening disease. Symptoms observed after 8-10 days of incubation are a flu-like manifestation with mild headache, muscle and joint pain. Occasionally, patients develop abdominal and gastrointestinal symptoms, but information about the real frequency of these manifestations is lacking⁶. While in secondary infection, DHF and DSS are manifested in more severe forms. The four important symptoms in WHO case definition of DHF are fever, thrombocytopenia, hemorrhage, and signs of plasma leakage. Undue emphasis laid on hemorrhage, while the hallmark manifestation is vascular permeability leading to plasma leakage into interstitial space, a characteristic feature of DSS. Occasionally, hemorrhage does not occur in severe dengue cases, it's an invariably late manifestation, which is linked by prolonged shock and death⁷.

Research suggests that shorter the time interval between sequential infection, the greater the protection, the latter is a severe manifestation⁵. On sequential infection, poorly neutralizing cross reactive antibodies signaled in response to a homologous serotype binds to the heterologous serotype, entry is mediated through the Fc-bearing receptors. Elevated levels of cross reactive antibodies enhance the antibody dependent enhancement (ADE) forming antibody-DENV complexes.

*Correspondent author
Email: srama@usm.my

Severe manifestations of dengue are rare but are more evidently occurring in younger people^{8,9}. Researchers have identified that primary infection with DENV2 or DENV4 do not lead to severe dengue cases on sequential infection, whereas primary infection by DENV1 or DENV3 manifest into severe cases on sequential infection. However, further investigations have proved that sequential infection with DENV2 was also associated with life-threatening dengue disease¹⁰.

Primary infection with a serotype gives lifelong immunity but sequential infection leads to ADE followed by severe disease. The development of the vaccine is targeted to be tetravalent vaccine, which can neutralize all four serotypes. Studies show that ADE is the primary mechanism for dengue immune-pathogenesis. Tetravalent vaccine candidates have to induce strong balancing immunity of all the four serotypes and minimize incomplete responses to make it to the market¹¹. No licensed vaccine against dengue is available in the market. This has initiated groups like; The Pediatric Dengue Vaccine Initiative (funded by Bill and Melinda Gates Foundation), the WHO and the US military, along with industries, governments and countries to succeed in the development of a licensed vaccine.

Experimental models are vital in the development of vaccines, especially when dengue is still complex and there are no accurate mechanisms or pathways elucidated to develop effective targeted dengue treatments. This is largely due to the lack of suitable animal models. Non-human primates (NHP) upon infection with dengue virus show increased viremia but lack clinical signs¹². Similarly, in immunocompetent mice, lack of viremia deems the model unsuitable. Therefore, it led to the development of immunodeficient mice models, these mice lack IFN receptors. The models are AG129, which lacks both Type I and II IFN receptors and A129, which lacks Type I IFN receptors. These two models are suitable to carry dengue vaccine testing as they recapitulate all the vital symptoms of dengue infection similar to humans. Therefore, this review will focus on the available mouse models for dengue vaccine testing and highlighting the advantageous and limitations of the available models for testing DENV-specific vaccines.

Virus structure

The dengue virus belongs to the Flaviviridae family, which includes Yellow fever virus (YFV),

West Nile virus (WNV), Japanese encephalitis virus (JEV), and Tick-borne encephalitis virus (TBEV). Flaviviruses are positive sense RNA molecules. Dengue virus has a positive RNA genome, which is approximately 10.6 kb long. Flanked by 5' to 3' untranslated region, a polyprotein precursor is encoded by the open. The polyprotein precursor is cleaved by cellular and viral protease contributing three structural proteins: nucleocapsid protein (C), precursor membrane proteins (PrM), and outer envelope protein (E). Seven non-structural proteins also exist, namely NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5^{13,14}.

The envelope glycoproteins facilitate for virus binding and entry, it is also responsible for neutralizing and enhancing antibodies. The glycoprotein is approximately 55 kDa and x-ray crystallographic studies reveal three distinct domains. Domain I is located in the center. Domain II has an internal fusion loop at the tip and participates in the membrane fusion and dimerization of E protein. Domain III, an immunoglobulin-like domain, is believed to be involved in receptor binding¹³⁻¹⁵. Domain II is very important as it consists of flavivirus group and sub-group cross-reactive epitopes. This is an important finding, as cross-reactivity of non-neutralizing anti-E antibody has been attributed to a higher risk of DHF/DSS upon secondary infection with a heterologous serotype^{13,15}.

Host response to dengue virus infection

Primary infection with the dengue virus manifests into a milder self-limiting form of the disease with symptoms such as fever, headache, nausea, myalgia, and vomiting. The host response to primary infection is formation of antibodies against that serotype. It has been discovered upon secondary infection with the same serotype, the host confers lifelong immunity due to these homotypic antibodies. Concurrently, cross reactive antibodies are produced against non-infecting virus serotypes¹⁶. In case of a secondary infection with a different serotype, the disease manifests into the more severe form of dengue called DHF/DSS; symptoms associated are elevated hematocrit, pleural effusion, hemorrhagic manifestations, thrombocytopenia, and shock¹⁷. Upon secondary infection, the cross reactive antibodies or heterotypic antibodies produced during primary infection only provide short term protection, as their levels decrease they facilitate replication of these viruses in monocytes via the FcγR receptors. This was identified

in vitro and this occurrence was termed as antibody-dependent enhancement¹⁸. Therefore, we could say that frequency of disease severity can be seen in people who have these heterotypic antibodies¹⁹. Primary dengue infections of infants with passively acquired dengue antibodies result in higher rates of severe disease than do secondary dengue infections²⁰. Furthermore, there are other factors that could play a role in disease severity such as genetic factors, viral virulence, and immunity of the host. Response to the infection could also depend on several other host and viral factors^{21,22}.

Need for an animal model

The prevalence of four different serotypes of the dengue virus makes it a unique disease. Disease prevalence is high due to the ever growing mosquito habits and the diversity of these serotypes; this indicates the complexity of the dengue virus. As dengue is a mosquito-borne human virus, disease manifestations occur only within the humans²³. The treatment regimens for dengue are currently symptom based and supportive care. The lack of appropriate animal models has been a major drawback towards research on dengue pathogenesis. Some non-human primates infected with these dengue pathogens show high viremia, presenting some degree of clinical signs. This led to the discovery of developed mouse models to study the virus pathogenesis²⁴. Immunocompetent mice were tested and they exhibited limited viremia¹², which led to the discovery of immunodeficient mouse models, and DENV infection of mice lacking both type I and II interferon (IFN) receptors or type I IFN receptor alone restructures the hallmark symptoms of dengue infection in humans.

Mouse models for dengue infection

Intracerebral infection with mouse-brain-adapted DENV

There were no signs of replication and disease manifestation within wild type mice infected with the dengue virus peripherally. Nevertheless virulent dengue virus in mice was developed by serial intracerebral passaging in suckling mice (6-10 days old mice)^{25,26}. The developed virus causes partial paralysis in mice over a long period of incubation. The manifestation of the disease isn't similar to that in humans and the route of virus entry isn't relevant to humans. The involvement of the nervous system in dengue virus infection is controversial and a rarity²⁷,

therefore limiting the scope of this animal model for dengue testing. In view with the guidelines set by WHO, this model is considered to be inappropriate for vaccine testing¹⁷.

Mouse-human chimera

The term mouse-human chimera refers to mice transplanted with human stem cells. This developed model has shown signs of fever and thrombocytopenia when infected with the dengue virus. Testing of vaccines can be undertaken in this chimeric model by evaluating the viral titers²⁸. A model of severe combined immunodeficient (SCID) mice transplanted with human liver cells (SCID-HuH-7) has been developed. This chimera proved the models efficacy in vaccine testing upon virus infection. The NOD/SCID/IL-2 gamma receptor-null mice reconstituted with human CD34+ cells have been used in DENV studies and demonstrate fever and thrombocytopenia²⁹.

Chimeric models are advantageous as they are able to simulate all the symptoms similar to human dengue infection. Interaction between human cells and the virus can be studied systemically when compared to the intracerebral infection with mouse-brain-adapted dengue virus model. Nevertheless, development of this chimeric model is tedious and variations from mouse to mouse are seen³⁰, which makes data interpretation difficult. In addition to that, several key molecules that are present during dengue infection are absent or not developed in this chimeric mouse model impacting virus cell interaction. Few such mechanisms that are absent are B cell maturation and antibody production³¹, and the coagulation cascade³². Due to these alterations and the absence of the hallmark symptom of human disease (vascular leakage), this is not the ideal model toward the dengue virus pathogenesis and immunity.

Interferon receptor-deficient mice

The dengue virus interferes with the type I and type II IFN receptor signaling in humans, whereas in wild type mice infection with dengue virus mimics no such interferences³³. The disruption of these IFN receptors in mice facilitates viral replication within mice and mimics the signs of severity of the disease in humans. Researchers have developed a mouse model with the absence of type I and type II IFN receptors (AG129), this model exhibits all the signs of severe DHF/DSS disease manifestation similar to humans³⁴. This model recreates all the hallmark symptoms of DHF such as

low platelet counts, vascular leakage, cellular and tissue tropism, increased hematocrit levels and hemorrhage³⁵. The ADE of the dengue virus can be clearly demonstrated in a mouse model that lacks the type I IFN receptor; the absence of these receptors causes dengue-specific antibody concentration to decrease, thus resulting in disease severity³⁶. This model has also been advantageous in elucidating the protective character of CD 4+ T cells in primary dengue infections and also the CD 4+ and CD 8+ T cells in vaccine developmental studies^{37,38}. This receptor knocked out model was initially developed using mouse adapted dengue virus 2 strain^{35,37,38}. Thereafter, the model was developed using dengue virus 2 strain, which wasn't mouse adapted³⁹.

The presence of IFN receptors within the immunocompetent mice makes the task of elucidating disease characteristics challenging, although the immunocompetent models would better clarify the protective and pathogenic host response to the dengue virus. The absence of the IFN receptors in the AG129 mice makes it a suitable model for viral replication as it mimics the exact environment in humans. The virus has an added advantage to replicate within these models that lack the IFN receptors not only in the infected cells but all the cells, which is not the case in human infection. Hence, care should be taken when the results obtained from these models are paralleled with human disease in developing vaccines.

Among the two models available, AG129 and immunocompromised A129, it is clear that the AG129 is a better model and is widely used by researchers to carry out antiviral testing. The advantage of AG129 mice model is that it is completely characterized and a low titer of virus is sufficient to manifest the signs of DHF/DSS and these doses are comparable to human infectious dose titers⁴⁰, when compared to A129 model, which requires higher doses to manifest DHF/DSS symptoms. The models that lack both the Type I and Type II IFN receptors are considered less suitable when compared to the immunocompromised A129 single-deficient receptor Type I (IFNAR^{-/-}) mice. In models lacking both the receptors, virus replicates irrepressibly in the nervous system, which could be attributed to the lack of the IFN γ pathway. Paralysis is seen in these mice after the symptoms of the acute disease have passed. As stated earlier, the effects of the dengue virus on the neuronal circuits is

questionable and controversial, therefore researchers don't consider death by paralysis on day 10 post infection as a characteristic feature during vaccine trials and antiviral testing seen in these models⁴⁰. Another disadvantage of the AG129 mice models are that lack of the IFN γ production (which is the major effector function of T cell) is not quantified. Therefore, the single-deficient receptor model is well suited as integral IFN γ pathway prevents viral replication in the neuronal circuits and prevents death by paralysis in these models post infection and also effector function of T cells can be measured. This makes this model ideal for testing immune pathways and the role IFN γ in dengue infection, thus it can be suggested that this mouse model will be the ideal candidate for vaccine and antiviral testing⁴¹.

The single-deficient receptor model, though lacking the Type I receptor still manages to show high B and T cell responses. Also, this model is used in studies related to specificity of the anti-dengue virus T cell responses⁴². IFNAR^{-/-} HLA transgenic mice can be employed to link T cell receptor findings made in IFNAR^{-/-} mice to the human situation⁴². HLA transgenic mice could be an ideal model to study the T cell responses. There are several variations in the T cell repertoire of humans in comparison to murine models¹²; this gap can be bridged by these HLA transgenic mice models as they are legitimately precise human models for T cell responses, especially when peptide immunizations are applied. Several new T cell epitopes have been identified using these transgenic mice⁴². Therefore, these models are ideal candidates for research studies carried out on vaccine trails and elucidating mechanism of virus pathogenesis.

Conclusion

The intracerebral model doesn't clearly explain the disease manifestation in mice due to the mode of viral entry and the effects on the neuronal circuits, which is questionable and deems this model invalid. The chimeric models are handy in simulating the disease states similar to those in humans but the presence of receptors in these models hinders complete understanding of this model. Therefore, interferon deficient mouse models are the preferred candidates for testing, the AG129 models could be used to elucidate the effects of vaccines in the absence of both the Type I and Type II IFN receptors, but lack of the IFN γ pathway shows the effects of virus replication in

neuronal circuits and the effector function of T cells is questionable in regards to dengue infection. In contrast, A129 single-deficient receptor (Type I) model is advantageous in elucidating the effects of the IFN γ pathway and the importance of the pathway and is preferred model for vaccine trails. HLA transgenic mice are advantageous in elucidating the T cell responses comparable to dengue infection in humans. The less stringent single-deficient receptor (IFNAR $^{-/-}$) mouse model will be necessary for determining efficacy of vaccines that are dependent on the IFN γ pathway.

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