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Mouse models of dengue virus infection and disease

Lauren E. Yauch and **Sujan Shresta***
Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037, United States

Abstract

Dengue virus (DENV) causes the most significant mosquito-borne viral disease in the world in terms of illness, death, and economic cost, due to the lack of an approved vaccine or antiviral. Infections with one of the four serotypes of DENV (DENV1–4) can result in diseases ranging from an acute, self-limiting febrile illness (dengue fever, DF) to life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), yet exactly how viral and host factors contribute to the severe disease is unknown. Clinical observations have provided information on DENV pathogenesis, but the lack of an adequate animal model has hindered research on this important human pathogen. A mouse model is ideal for investigating host–pathogen interactions due to the immunological tools available, however, wild-type mice are resistant to DENV-induced disease. Therefore, the mouse models for DENV infection developed to date include infection of severely immunocompromised mice, non-physiologic routes of infection, and mouse–human chimeras, which all have their limitations. An inbred mouse model in which mice develop signs of human DENV-induced disease is needed to investigate the contribution of various immune components to protection and pathogenesis of DENV infections, and to test the efficacy of DENV vaccines and antivirals.

Keywords

Flavivirus; Dengue virus; Mouse model; Vaccine; Antiviral therapy; Dengue fever; Dengue hemorrhagic fever/dengue shock; syndrome

1. Introduction

Dengue virus (DENV) belongs to the Flaviviridae family of enveloped, positive-strand RNA viruses, and is transmitted by the mosquitoes *Aedes aegypti* and *Aedes albopictus*. Humans are the primary vertebrate host for DENV, and infections occur in the tropical regions of Asia, Oceania, Africa, and the Americas (McBride and Bielefeldt-Ohmann, 2000). Globally, cases of dengue fever (DF) and dengue hemorrhagic fever (DHF) have been increasing over the past 50 years, and 2.5 billion people are now at risk of infection (Gubler, 1998). The single-stranded genome of DENV is approximately 10.7 kb and encodes three structural (core (C), envelope (E), and membrane (M)) and seven non-structural (NS) (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) proteins (Chambers et al., 1990). The four DENV serotypes (DENV1–4) are 67–75% homologous at the amino acid level (Fu et al., 1992).

Humans become infected when fed on by infected mosquitoes, which presumably deposit the virus into the dermis and blood. Skin-resident dendritic cells (DCs) may be the initial targets of the virus (Wu et al., 2000). DENV enters target cells by receptor-mediated

*Corresponding author. Tel.: +1 858 752 6944; fax: +1 858 752 6987. sujan@liai.org (S. Shresta).

endocytosis via a direct interaction of the viral E glycoprotein with host cell receptors, including DC-specific ICAM-3-grabbing non-integrin (DC-SIGN) (Tassaneetrithep et al., 2003; Lozach et al., 2005) and the mannose receptor (Miller et al., 2008). Some cells can also be infected in the presence of DENV-specific antibody, in a process termed antibody-dependent enhancement (ADE) of infection, by which antibody-opsonized virus is internalized via immunoglobulin Fcγ receptor (FcγR) expressing cells, such as macrophages (Morens et al., 1987). DENV antigens have been detected in monocytes, lymphocytes, Kupffer cells, alveolar macrophages, and endothelial cells of DENV-infected humans (Jessie et al., 2004). However, the identity of the cell types that are productively infected in vivo is still unclear.

2. DENV-induced disease

The majority of symptomatic DENV infections present as DF, which is a debilitating febrile illness. Viremia lasts for 2–12 days, and high viral titers in the blood can be reached (10^3 to $10^{8.5}$ mosquito infectious doses/ml) (Gubler, 1998). Symptoms of DF include fever, rash, headache, retro-orbital pain, muscle and joint pain, nausea and vomiting, and can include hemorrhagic manifestations (WHO, 1997). Elevated liver enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) are common in DF and DHF/DSS (dengue shock syndrome) cases. A diagnosis of DHF, based on World Health Organization (WHO) definitions, requires the presence of fever, hemorrhagic tendencies, thrombocytopenia, and plasma leakage. Hemorrhagic tendencies are indicated by a positive tourniquet test, petechiae, bleeding from the mucosa, gastrointestinal (GI) tract, or injection site, hematemesis (vomiting of blood), or melaena (black feces due to GI hemorrhage). A diagnosis of DSS requires all of the criteria listed for DHF, and in addition, signs of circulatory failure, including rapid and weak pulse or hypotension. DHF/DSS is classified into four grades of severity (I–IV), with grades III and IV denoting DSS. DF is self-limiting, but DSS can be fatal if patients do not receive fluid replacement.

3. Immune response to DENV

Infection with one DENV serotype presumably results in lifelong immunity to that serotype, but does not confer immunity to the other serotypes. In fact, DHF/DSS is most often observed in individuals experiencing a secondary infection with a heterologous serotype (Sangkawibha et al., 1984; Burke et al., 1988). It has been postulated that cross-reactive antibodies and T cells are involved in the pathogenesis of secondary infections. As such, studies to date have focused on the role of the adaptive immune system in the pathogenesis of secondary infections. Because of the lack of a good mouse model, the majority of these studies were done using patient samples and were therefore descriptive in nature.

In vitro studies have demonstrated that non-neutralizing concentrations of serotype cross-reactive, DENV-specific antibodies enhance viral replication, suggesting that antibodies produced during previous infection or passively acquired contribute to DHF/DSS via ADE (Kliks et al., 1989; Morens and Halstead, 1990; Goncalvez et al., 2007). In support of the ADE hypothesis, almost all DHF/DSS patients have DENV serotype cross-reactive antibodies (Burke et al., 1988), and infants with DHF/DSS due to primary infection have mothers with DENV-specific antibodies (Halstead, 1988). In addition to antibodies, cross-reactive T cells may also contribute to the immunopathogenesis of secondary infections. It is hypothesized that low-affinity T cells raised against the original infecting serotype dominate during a secondary heterologous infection in a phenomenon termed ‘original antigenic sin’ (Mongkolsapaya et al., 2003). Human studies have found that serotype cross-reactive T cells are preferentially activated during secondary infection, and these cross-reactive T cells exhibit suboptimal degranulation and altered cytokine production (Mongkolsapaya et al.,

2003, 2006; Mangada and Rothman, 2005; Imrie et al., 2007). Activation of cross-reactive memory T cells likely contributes to severe disease via the activation of innate immune cells and enhanced cytokine production. Cytokines, such as tumor necrosis factor α (TNF-α), may play a pathogenic role in DHF/DSS by causing endothelial cell dysfunction and subsequent plasma leakage, which is a hallmark of DHF/DSS. Indeed, TNF-α has been detected more frequently and at higher levels in the serum of patients with DHF/DSS than patients with DF (Green et al., 1999; Mangada et al., 2002).

In contrast to studies examining immunopathogenesis of DENV infection, little is known about what constitutes a protective anti-DENV response. Interferon (IFN)-dependent immunity, which includes IFN-α, IFN-β, and IFN-γ, plays a critical role in the host response against DENV (Johnson and Roehrig, 1999; Diamond et al., 2000). Neutralizing antibodies are also important in protecting against re-infection with the same DENV serotype, however, the contribution of T cells to protection remains unknown. A genetically tractable mouse model is required to determine how the various immune system components orchestrate a protective immune response, and to investigate how cross-reactive antibodies and T cells contribute to severe disease during secondary heterologous infection.

4. Status of DENV vaccines and antivirals

There is currently no approved vaccine or specific antiviral available for DENV infections. Several vaccines are under development. The status of DENV vaccines has recently been covered in an excellent review by Whitehead et al. (2007). A DENV vaccine must provide immunity against all four serotypes, so as to avoid induction of ADE. DENV vaccines that are in various stages of development include live attenuated virus, inactivated virus, subunit, vectored, and DNA vaccines. Mice have been used to test vaccine candidates prior to testing in nonhuman primates. However, these models have measured protection from lethal DENV encephalitis, which is not relevant to human disease, as discussed below.

A number of antiviral compounds have shown efficacy in inhibiting DENV replication in vitro, including ribavirin, mycophenolic acid, 7-deaza-2′-*C*-methyl-adenosine (7-DMA), *N*-nonyl-deoxynojirimycin (NN-DNJ), and 6-*O*-butanoyl castanospermine (Courageot et al., 2000; Diamond et al., 2002; Wu et al., 2002; Olsen et al., 2004; Whitby et al., 2005; Takhampunya et al., 2006). Anti-DENV effects have been found for the α-glucosidase inhibitors castanospermine and NN-DNJ, and 7-DMA, which inhibits RNA-dependent RNA polymerase, in mouse models of DENV as discussed below (Whitby et al., 2005; Schul et al., 2007). Antisense RNA strategies are also being tested for their ability to inhibit DENV replication in vitro, and will need to be tested in vivo (reviewed in Stein and Shi, 2008). Thus, a better mouse model in which to test DENV-specific vaccines and antivirals is desired.

5. DENV infection of nonhuman primates

The known natural hosts for DENV are humans and mosquitoes. Serological evidence from primates also supports the existence of a sylvatic cycle (Wang et al., 2000). Chimpanzees and several species of monkeys have been infected with DENV via the subcutaneous (s.c.) route (Halstead et al., 1973b; Scherer et al., 1978). Although these primates develop viremia and a neutralizing antibody response, there is only limited evidence of disease or hematologic abnormalities as seen in humans, apart from one study in rhesus macaques that demonstrated leukopenia after a primary DENV infection and increased thrombocytopenia after a secondary, heterologous infection (Halstead et al., 1973a). Viremia in macaques begins 2–6 days after s.c. infection and lasts for 3–6 days. DENV rapidly spreads from the inoculation site to the regional lymph nodes, and during the viremic period can be isolated

from the skin, distant lymph nodes, and rarely from the spleen, thymus, liver, lung, and bone marrow (Marchette et al., 1973).

Nonhuman primates have been used to study ADE (Halstead, 1979; Goncalvez et al., 2007) and to test the efficacy and safety of candidate vaccines (Guirakhoo et al., 2002; Blaney et al., 2005; Sun et al., 2006; L. Chen et al., 2007). In the vaccine studies, antibody titers and T cell responses were measured, and protection indicated by reduced/absent viremia. While nonhuman primates are needed to test potential vaccines/antivirals prior to human clinical trials, this model is too prohibitive in terms of cost and accessibility to be used to answer basic questions about DENV pathogenesis.

6. Established mouse models of DENV infection

The difficulty in developing a mouse model for DENV is largely the result of the inability of human clinical isolates to replicate well in mice. Although a variety of mouse models have been developed, the majority are not ideal for investigating mechanisms of pathogenesis. The models include intracerebral (i.c.) infection, infection with mouse-brain-adapted DENV strains that predominantly target the central nervous system (CNS), chimeric mice transplanted with human cells, and severely immunocompromised mice (Table 1).

6.1. Intracerebral infection of mice with mouse-brain-adapted DENV

In order to adapt this human pathogen to mice, DENV isolates have been inoculated intracerebrally into suckling mice, and then serially passaged from brain to brain. Passaging the virus resulted in increased neurovirulence in mice (Sabin and Schlesinger, 1945; Cole and Wisseman, 1969) and attenuation in human volunteers (Hotta, 1952; Sabin, 1952). This mouse model has been predominately used to test the efficacy of DENV vaccines (Kaufman et al., 1987; Bray et al., 1989; Falgout et al., 1990; van Der Most et al., 2000). These studies have identified a number of immunization strategies that can protect mice from lethal DENV-induced encephalitis; however, this disease manifestation is not relevant to human dengue disease, as nervous system involvement in DENV infections is rare (Patey et al., 1993; Lum et al., 1996).

6.2. Mouse–human chimeras

To obtain a mouse model in which DENV can be administered by peripheral inoculation (i.e. not into the CNS), a number of mouse–human chimeras have been developed. Severe combined immunodeficient (SCID) mice, which lack T and B cells, are not susceptible to DENV infection, but become permissive following engraftment with some types of human cells. SCID mice have been engrafted with human peripheral blood lymphocytes (SCID-hu-PBL mice) and subsequently infected intraperitoneally (i.p.). with DENV1 (Wu et al., 1995). Only a small percent of the mice supported the DENV infection and had detectable virus in the serum, possibly due to variable levels of human cell engraftment or limited infection of lymphocytes. In another model, SCID mice were engrafted with human K562 erythroleukemic cells by i.p. injection (Lin et al., 1998). Following intratumor injection of 10^7 PFU DENV2, the mice demonstrated virus in the blood, tumor, and brain. Serum titers peaked around day 6 after infection, and titers in the brain increased from day 3 onwards. The mice began to show signs of paralysis 1–2 weeks post-infection and died 2–4 weeks post-infection.

SCID mice have also been engrafted with human liver cells, as liver involvement is common in DENV infections (Seneviratne et al., 2006). SCID mice were engrafted with human HepG2 cells (a hepatocarcinoma cell line) which then propagated in the liver (An et al., 1999). Following i.p. infection with DENV2, the SCID-HepG2 mice had detectable virus in the serum, liver, and brain, but not spleen, lung, or small intestine. The SCID-HepG2 mice

developed paralysis 13–18 days post-infection, at which time they exhibited thrombocytopenia, an elevated hematocrit, and increased serum TNF-α levels, which are associated with DHF/DSS. In a similar model, SCID mice were engrafted with HuH-7 human hepatoma cells (Blaney et al., 2002). Intratumor injection of these SCID-HuH-7 mice with DENV4 resulted in a productive infection, with detectable virus in the serum, liver, and brain. This model has since been used to test DENV vaccine candidates (Whitehead et al., 2003; Blaney et al., 2005).

Nonobese diabetic (NOD)/SCID mice are suitable for human cell engraftment due to their lack of T cells, B cells, the complement component C5, and defective natural killer (NK) cell and antigen-presenting cell functions. These mice engrafted with $CD34^+$ human cord blood hematopoietic progenitor cells were susceptible to s.c. infection with DENV2 (Bente et al., 2005). Viremia peaked 2–6 days after infection, and viral RNA was detected in the spleen, liver, and skin of some mice. Reconstituted mice developed clinical signs of DF, including fever, rash, and thrombocytopenia. Both fever and rash are useful clinical indicators for DENV-induced disease, as they can be assessed in a non-invasive manner. More recently, in a similar model, neonatal T-, B-, and NK-cell-deficient ($RAG2^{-/-}$ $\gamma_c^{-/-}$) mice transplanted with human fetal liver-derived $CD34^+$ cells were also shown to support DENV2 infection (Kuruvilla et al., 2007). In this model, reconstituted mice infected with various combinations of DENV2 strains via i.p. plus s.c. routes demonstrated viremia lasting up to 21 days, fever that peaked on day 8 post-infection, and DENV-specific human IgM and IgG responses.

These mouse–human chimeric models have provided insight into pathogenesis and have been useful for vaccine studies evaluating virus attenuation in vivo. The models that manifest viremia and symptoms of human DENV disease, including fever and thrombocytopenia, may be used to test DENV antivirals and vaccines. However, these xenografted mice have their limitations. Engraftment of human cells into mice is advantageous in that the response of human cells can be analyzed, but crosstalk between various components of the immune system may be altered in a mouse–human chimera. Furthermore, chimeric mice possess substantial inter-host variability due to variable levels of human cell engraftment, and creation of these mice is laborious. These models are thus not optimal for investigating the immune response to DENV.

6.3. Immunocompromised mice

Immunodeficient mouse strains have demonstrated varying susceptibilities to infection with DENV. BALB/c athymic nu/nu mice and heterozygote littermates exhibited 40–60% mortality following i.p. infection with the mouse-brain-adapted DENV1 strain, Mochizuki (Hotta et al., 1981). $RAG1^{-/-}$ mice, which lack T and B cells, are susceptible to infection with a DENV2 clinical isolate, PL046, with 31% mortality (Shresta et al., 2004b). In these models, death resulted from paralysis.

Because of the importance of the IFN system in the host antiviral response, mice lacking both IFN-α/β and IFN-γ receptors on the 129/Sv background (AG129) were tested for their susceptibility to DENV infection. Following i.p. infection with the mouse-brain-adapted DENV2 strain, New Guinea C (NGC), 100% of AG129 mice developed paralysis (Johnson and Roehrig, 1999). Similarly, 100% of AG129 mice died following intravenous (i.v.) infection with PL046 (Shresta et al., 2004b). In these studies, virus was detected in the serum and spleen early post-infection, but titers increased in the brain and the mice developed paralysis. AG129 mice have been used to investigate cellular tropism of DENV (Kyle et al., 2007) and to test vaccine candidates (Calvert et al., 2006). These mice were also found to be a good model to test antiviral drugs (Schul et al., 2007). Administration of inhibitors of the viral RNA-dependent RNA polymerase or host α-glucosidase resulted in

lower viremia following DENV2 infection. Single-deficient mice lacking either the IFN-α/β or the IFN-γ receptor on the 129/Sv background (A129 and G129, respectively) were found to be less susceptible to infection with PL046 than the AG129 mice (Shresta et al., 2004b). Again, a major limitation of these models involving immunocompromised mice is that paralysis is not a relevant phenotype. The neurotropism of DENV in mice, even when the mice are infected by non-mouse-brain-adapted viruses, and/or by an extraneural route, is interesting. However, other members of the Flaviviridae family including West Nile virus, Japanese encephalitis virus, and tick-borne encephalitis virus are neurotropic and cause encephalitis in humans.

A/J mice, which lack the complement component C5, infected i.v. with a high inoculum of PL046 exhibited viremia at early time points post-infection and transient thrombocytopenia at later time points (Huang et al., 2000). A subset (71%) of the A/J mice developed paralysis following infection. Another study found that the same high i.v. dose of PL046 resulted in an elevated hematocrit, decreased white blood cell counts, and activation of natural killer cells and B cells (Shresta et al., 2004a). A/J mice were also used to demonstrate the antiviral effect of the α-glucosidase inhibitor, castanospermine, on DENV-induced encephalitis (Whitby et al., 2005).

In order to establish a more relevant mouse model that does not target the CNS, our laboratory passaged a DENV isolate through the serum of mice and mosquito cells, mimicking the natural transmission cycle (Shresta et al., 2006). Briefly, AG129 mice were infected i.v. with the DENV2 strain PL046, and 3 days later their serum was harvested and virus amplified in mosquito cells. This was repeated a total of 10 times, and the virus isolated at the end was termed D2S10. Instead of the paralysis observed in PL046-infectedmice,D2S10-infectedAG129micediedearlypost-infection and demonstrated increased TNF-α levels and vascular permeability.

D2S10 is also more virulent than the parental strain, PL046, in IFN-α/β receptor-deficient mice. IFN-α/β$R^{-/-}$ mice on the C57BL/6 background infected i.v. with a biological clone from the D2S10 population, S221, show signs of disease (hunched posture and ruffled fur) and have virus in the serum, spleen, and brain at early time points after infection, and recover around day 10 (our unpublished observations). These mice may be a better model than the AG129 mice, as they are less immunocompromised. Future studies will determine if these mice also demonstrate the hallmarks of severe dengue disease, including thrombocytopenia, increased vascular permeability, and elevated TNF-α levels.

Also less compromised than the AG129 mice are $STAT1^{-/-}$ mice, which lack a transcription factor involved in IFN signaling (Durbin et al., 1996). $STAT1^{-/-}$ mice were recently used to demonstrate a role for the C-type lectin, CLEC5A, in DENV-induced vascular leakage and hemorrhage (Chen et al., 2008). The mice were infected with DENV2 NGC via the i.p. plus i.c. routes, and exhibited ruffled fur, mild paralysis and subcutaneous and intestinal hemorrhage 8 days after infection and vascular leakage at day 9. Although the immunocompromised mouse models have given insight into DENV pathogenesis, they are not ideal for analysis of the immunocompetent host response to DENV.

6.4. Immunocompetent mice

While the use of mice lacking IFN signaling revealed the importance of IFNs in a protective immune response against DENV, an obvious limitation of this model is that it is difficult to study the immune response to DENV in an animal that lacks a critical component of the host antiviral system. Ideally, non-immunocompromised mice should be used to investigate the immune response to DENV and to test DENV vaccines and antivirals. Although wild-type mice are relatively resistant to DENV-induced disease, they have been used to investigate

some aspects of DENV pathogenesis. Recently, H.C. Chen et al. (2007) induced hemorrhage in a subset of wild-type DENV-infected mice. Intradermal (i.d.) infection of C57BL/6 mice with the non-mouse-adapted DENV2 strain, 16681, resulted in hemorrhage: a high inoculum resulted in systemic hemorrhage and a lower dose caused subcutaneous hemorrhage. The mice that developed hemorrhage also had severe thrombocytopenia, and the combination of high DENV titers, TNF-α production, and macrophage infiltration contributed to endothelial cell apoptosis and the hemorrhagic manifestations. In a separate study, i.v. infection of C57BL/6 mice with a high inoculum of DENV2 16681 also resulted in liver damage, another feature of DENV infections in humans (Chen et al., 2004). Similarly, BALB/c mice infected i.p. with DENV2 demonstrated liver damage, as determined by immunohistochemistry and elevated AST and ALT levels that peaked at day 7 post-infection (Paes et al., 2005). Collectively, these studies reveal that wild-type mice can exhibit various clinical signs of DENV infection, including thrombocytopenia, hemorrhage, and liver damage.

Studies examining immunity to DENV have shown that wild-type mice mount an anti-DENV T cell response. $CD4^+$ and $CD8^+$ T cell clones derived from DENV2 NGC-infected BALB/c mice recognize epitopes from the DENV proteins prM, E, NS1/NS2a, and NS3 (Rothman et al., 1996). $CD8^+$ T cell responses during secondary heterologous infections have been studied in wild-type mice as well (Beaumier et al., 2008). BALB/c mice were infected i.p. with one DENV serotype, followed by infection with the same or a heterologous serotype 28–56 days later. An enhanced $CD8^+$ T cell response (as measured by IFN-γ production in response to two DENV epitopes) was observed during certain secondary, heterologous infections depending on the sequence of infection. However, in these studies viremia was not measured and the T cell responses were low, which is dissimilar from DENV infection of humans. Therefore, it remains to be determined if $CD8^+$ T cells can contribute to the immunopathogenesis of secondary infections. Altogether, these studies indicate it is possible to investigate some aspects of DENV pathogenesis in immunocompetent mice. Nevertheless, wild-type mice do not support high enough levels of replication to easily dissect the protective versus pathogenic roles of the host response to primary or secondary DENV infections.

7. Development of new mouse models

Development of a mouse model for studying DENV pathogenesis has been and continues to be a challenge. Ideally, the model should be as physiologically relevant as possible in terms of the route of infection and the immunocompetence of the host, and should induce signs of disease in wild-type mice similar to those seen in humans. A model in which some mice develop a DF-like disease and some manifest symptoms of DHF/DSS following a secondary, heterologous infection is desired. The mouse model will be critical in elucidating the mechanism by which cross-reactive antibodies and T cells contribute to the pathogenesis of secondary infections. While it is often difficult to know the order of infecting serotypes in sequential human DENV infections, using a mouse model makes it possible to manipulate the sequence of infections.

The major obstacle to such a model is the lack of DENV strains capable of replicating well in wild-type mice. As discussed above, mice are largely resistant to DENV infection, in particular to infection with human clinical isolates. One way this has been overcome is by serially passaging the virus through mice, which increases virulence. While clinical DENV isolates passaged through the suckling mouse brain showed enhanced neurovirulence for mice, a DENV isolate that replicates in peripheral tissues (spleen, liver, intestine, etc.) was desired. By passaging a clinical isolate of DENV through the serum of mice and mosquito

cells, we established a more relevant model of DENV, as infection with D2S10 caused an early, TNF-α-mediated disease, as discussed above.

We are currently investigating whether the mutations in the envelope protein of D2S10 that are responsible for the virulent phenotype affect heparan sulfate binding. Heparan sulfate is a glycosaminoglycan (GAG) that serves as an attachment factor for many viruses, including DENV (Chen et al., 1997). It has been shown that passaging viruses in vitro can lead to altered GAG binding and subsequent attenuation in vivo (reviewed by Spillmann, 2001). For example, a variant of the DENV2 strain, PUO-218, that acquired increased affinity for GAGs by tissue culture passaging was found to be more rapidly cleared from the circulation (Lee et al., 2006). Thus, tissue culture-adapted DENV strains are likely not the most relevant to use in a mouse model. Also, it is probable that many clinical isolates of DENV have been passaged in vitro, either through mammalian cells or mosquito cells, in order to obtain a high enough titer to infect mice. Most DENV strains are not sequenced immediately after isolation from infected patients, and it is therefore unknown what mutations, if any, they have since undergone. The relative virulence of direct human clinical isolates versus mouse serum passaged DENV strains in mice remains to be determined. Ideally, DENV should be sequenced directly from patient samples, infectious clones made and then used to infect mice. If these viruses are not virulent in mice, mouse serum–mosquito cell passaging can be performed to increase their virulence.

Of all the routes of infection that have been used in mouse models of dengue (i.c., i.p., i.v., s.c., and i.d.), i.d. and s.c. routes in combination may best mimic the bite of an infected mosquito. However, it is likely that some virus is introduced directly into the bloodstream, as recently demonstrated for mice fed on by West Nile virus-infected mosquitoes (Styer et al., 2007), lending credence to the i.v. route of infection. Even more relevant may be i.d. co-inoculation of DENV and mosquito salivary gland extract, which has been shown to have immunomodulatory effects (Schneider and Higgs, 2008).

8. Conclusion

Much progress has been made in recent years in the development of a mouse model for DENV. Models in use today exhibit some features of human clinical disease, including viremia, fever, thrombocytopenia, hemorrhage, and elevated TNF-α levels. However, they still have limitations, including the immunocompetence of the mice and the laboriousness and variability associated with xenografting. Approaches involving infectious clone-derived viruses, alternate passaging between mosquito cells and mouse serum, and i.d. and/or. s.c. infection should lead to the generation of mouse models of primary and secondary DENV infections that are more relevant to human disease than are currently available.

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Table 1

Mouse Models of DENV Infection.

Mouse	DENV inoculation	Features/outcome	Reference
Mouse–human chimeras			
SCID-hu-PBL	DENV1 $9 \times 10^{4–5}$ PFU i.p.	Virus in serum of some mice	Wu et al. (1995)
SCID-K562	DENV2 10^7 PFU i.t.	Virus in tumor, serum, brain, lung, liver, kidney, spleen; paralysis	Lin et al. (1998)
SCID-HepG2	DENV2 10^6 PFU i.p.	Virus in serum, liver, brain, thrombocytopenia, elevated hematocrit, serum TNF-α; paralysis	An et al. (1999)
SCID-HuH-7	DENV4 10^4 PFU i.t.	Virus in serum, liver, brain	Blaney et al. (2002)
NOD/SCID-human $CD34^+$	DENV2 $10^{4.7}$ PFU s.c.	Virus in serum, liver, skin; fever, rash, thrombocytopenia	Bente et al. (2005)
$RAG2^{-/-}\gamma c^{-/-}$-human $CD34^+$	DENV2 10^6 IU i.p. + s.c.	Virus in serum; fever, human antibody response	Kuruvilla et al. (2007)
Immunocompromised			
AG129	DENV2 10^6 PFU i.p.	Virus in serum, spleen, brain; paralysis	Johnson and Roehrig (1999)
AG129	DENV2 (PL046) 10^7 PFU i.v.	Paralysis	Shresta et al. (2006)
AG129	DENV2 (D2S10) 10^7 PFU i.v.	Vascular leakage, early TNF-α-mediated death	Shresta et al. (2006)
A/J	DENV2 10^8 PFU i.v.	Virus in serum, thrombocytopenia, temporary paraplegia	Huang et al. (2000)
A/J	DENV2 10^8 PFU i.v.	Elevated hematocrit, decreased WBC	Shresta et al. (2004a)
$STAT1^{-/-}$	DENV2 10^5 PFU i.p. + i.c.	s.c. and intestinal hemorrhage, vascular leakage; paralysis	Chen et al. (2008)
Immunocompetent			
C57BL/6	DENV2 $4–8 \times 10^7$ PFU i.d.; DENV2 $1–3 \times 10^9$ PFU i.d.	s.c. hemorrhage, thrombocytopenia; systemic hemorrhage	H.C. Chen et al. (2007)
C57BL/6	DENV2 10^8 PFU i.v.	Liver damage	Chen et al. (2004)
BALB/c	DENV2 10^4 $TCID_{50}$ i.p.	Liver damage	Paes et al. (2005)

Abbreviations: PFU, plaque-forming unit; i.p., intraperitoneal; i.t., intratumor; s.c., subcutaneous; i.v., intravenous; IU, infectious units; WBC, white blood cell count; TCID, tissue culture infectious dose.