

Review Article



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Conflict of Interest

The authors declared no potential conflicts of interest.

Abbreviations

CNS, central nervous system; IFN, interferon; IRF, interferon regulatory factor; NHP, non-human primate; WT, wild-type; ZIKV, Zika virus

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Understanding the Pathogenesis of Zika Virus Infection Using Animal Models

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ABSTRACT

Zika virus (ZIKV) is a member of *Flaviviridae* family that has emerged as a pathogen of significant public health importance. The rapid expansion of ZIKV in the South and Central America has recently gained medical attention emphasizing the capacity of ZIKV to spread to non-endemic regions. ZIKV infection during pregnancy has been demonstrated to cause microcephaly and other fetal developmental abnormalities. An increased incidence of Guillain-Barre syndrome, an immune mediated neuropathy of the peripheral nervous system, has also been reported in ZIKV-infected patients in French Polynesia and Brazil. No effective therapies currently exist for treating patients infected with ZIKV. Despite the relatively short time interval, an intensive effort by the global scientific community has resulted in development of animal models to study multiple aspects of ZIKV biology. Several animal models have been established to investigate pathogenesis of ZIKV in adults, pregnant mothers, and developing fetuses. Here we review the remarkable progress of newly developed small and large animal models for understanding ZIKV pathogenesis.

Keywords: Zika virus; Animal model; Etiology

INTRODUCTION

Zika virus (ZIKV) is an emerging mosquito-borne pathogen that is part of the Spondweni serocomplex of the genus *Flavivirus*, family *Flaviviridae*. ZIKV is closely related to other pathogens of public health importance including yellow fever virus (YFV), dengue virus (DENV), Japanese encephalitis virus (JEV), and West Nile virus (WNV). The ZIKV genome is comprised of a single-stranded, positive-sense 11-kb RNA that contains three structural genes (capsid [C], precursor of membrane [M], and envelope [E]) and seven nonstructural genes (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (1).

Little is known about ZIKV pathogenesis, however it is thought that after an infected mosquito bite, viral replication occurs in local dendritic cells with subsequent spread to lymph nodes and the bloodstream. Viremia is generally seen within 3 to 4 days after onset of symptoms, and approximately 80% of individuals infected with ZIKV have no symptoms. Patients with symptomatic ZIKV infection usually present with a mild febrile illness characterized by fever, rash, arthralgia, myalgia, headache, and conjunctivitis (1,2). During

the first week after onset of symptoms, ZIKV infection can often be diagnosed by performing quantitative RT-PCR (qRT-PCR) on serum specimens. ZIKV-specific IgM and neutralizing antibodies typically develop toward the end of the first week of illness (1).

ZIKV was isolated in 1947 from the blood of a sentinel rhesus monkey in the Zika forest of Uganda. ZIKV caused only sporadic cases of infection in Africa and Southeast Asia until 2007, when the first large outbreak occurred on the island of Yap in the Federated States of Micronesia. Another outbreak in French Polynesia in 2013 was notable for being associated with an increase in cases of Guillain-Barré syndrome (1,2). In 2015, the virus was first reported in Brazil and since then has spread through several additional countries in South and Central America and the Caribbean. ZIKV outbreaks have also been recorded in the United States (3-7). Simultaneously, several of these countries have seen a dramatic increase in the incidence of infants born with microcephaly (1,2,8-10).

During the recent epidemic in Latin America, ZIKV infection has been linked to the development of severe fetal abnormalities that include spontaneous abortion, stillbirth, hydranencephaly, microcephaly, and placental insufficiency that may cause intrauterine growth restriction (1,8,11). Unlike most other flaviviruses, ZIKV has the potential for significant human-to-human transmission through sexual and vertical routes (9,10,12). Phylogenetic analysis of ZIKV genomes reveals African and Asian strains of ZIKV as two distinct lineages (1,13). The African lineage viruses have caused sporadic human infections in the last century, resulting in mild, febrile disease symptoms (1,14-17). The Asian lineage has however emerged at a larger scale displaying vector-borne as well as human-to-human transmission, causing fetal abnormalities and neuronal disease in humans (1,8,18-20). Comparison of isolates from Brazil and French Polynesia show 87% to 90% sequence similarity to the original MR 766 strain from Uganda (13).

No effective therapies currently exist for treating patients infected with ZIKV. Recently several drugs and therapeutic candidates have been evaluated in cell culture and animal models. These anti-ZIKV drugs include drugs targeting virus entry into the cells and helicase protein, inhibitors of NS3 protein, methyltransferase inhibitors, and interferons (IFNs). The viral polymerase inhibitor 7-Deaza-2'-C-methyladenosine (7DMA) has been demonstrated to efficiently inhibit ZIKV replication, reduce viremia, and delay ZIKV-associated morbidity and mortality in a mouse model (21). Sofosbuvir, an RdRp inhibitor approved by the US Food and Drug Administration for the treatment of hepatitis C virus (HCV) infection, efficiently inhibits infection and replication of several ZIKV strains in human cells and isolated neuronal stem cells (22-24). Similarly, sofosbuvir treatment protects mice against ZIKV-associated morbidity and mortality. The small molecule drug candidate BCX4430, a broad-spectrum antiviral, has also been demonstrated to be protective against ZIKV-associated mortality in a mouse model (25). In addition, several reports demonstrated anti-ZIKV activities of T-705 (21,26-29). T-705 (favipiravir) is a novel antiviral compound that selectively and potently inhibits the RdRp common to several RNA viruses, including influenza virus (30-32). Currently, there are no licensed vaccines for ZIKV prevention. World Health Organization has announced that ZIKV vaccine development is a top priority, and various approaches are being tried including inactivated virus, live attenuated virus, recombinant E protein, virus like particle, DNA vaccine, mRNA based vaccine and peptides (33). Purified inactivated ZIKV vaccine has been shown to be effective against ZIKV challenge in both mice and rhesus monkeys (34,35). Griffin et al. (36) has reported that a DNA vaccine coding ZIKV prM-E protects mice against ZIKV-associated tissue damage.

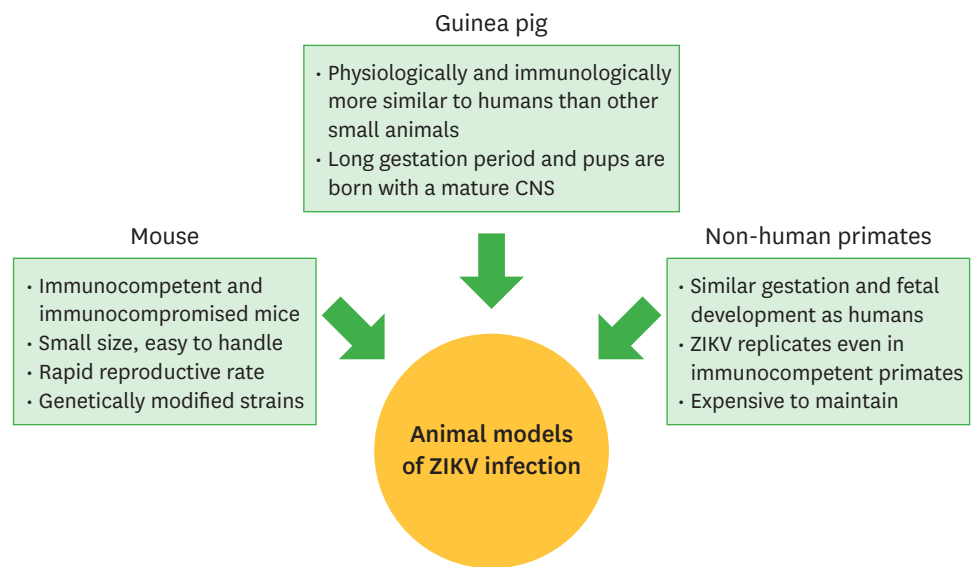


Figure 1. Characteristics of animals models of ZIKV infection.

ANIMAL MODELS OF ZIKV INFECTION

The factors that contributed to the emergence, global spread of ZIKV and change in the disease phenotype are not well understood. Animal models of ZIKV infection and disease are critical to study ZIKV pathogenesis, including pregnancy outcomes and evaluation of vaccines and therapeutics. Recently, ZIKV infection and disease have been evaluated in non-pregnant and pregnant animals, as well as a large panel of immunodeficient transgenic mice. Here we summarize the animal models that have been used to study ZIKV pathogenesis and to develop vaccine and therapeutic strategies (**Fig. 1**).

SMALL ANIMAL MODELS OF ZIKV INFECTION

Immunocompetent mouse models

Prior to the recent ZIKV epidemics, few animal models of ZIKV infection existed. From the first isolation of ZIKV in 1947 until 2015, there were only three studies which tested the virus' pathogenic potential in animal models (37-39). In the first publication on ZIKV infection in a mouse model, inoculation of ZIKV via intracranial route caused neurological disease in suckling or adult mice (37). However, infection of adult immunocompetent mice with ZIKV via intraperitoneal route did not cause disease, indicating that intracranial route of inoculation is necessary to establish any successful infection. This study used the prototype MR 766 strain of ZIKV, which had undergone extensive passage in suckling mouse brains (40). Recently, ZIKV infection and disease have been evaluated in non-pregnant and pregnant immunocompetent mice using contemporary ZIKV strains. After peripheral (subcutaneous, intra-peritoneal, and intravenous) ZIKV inoculation, no clinical disease and little or no virus was detected in wild-type (WT) C57BL/6, Swiss Webster, BALB/c, and CD-1 mice (34,41,42). Many different strains of ZIKV have been examined in similar mouse studies and regardless of the strain of ZIKV, similar results have been reported (43). Similarly, no fetal defects were observed after peripheral inoculation of ZIKV into pregnant WT C57BL/6 mice (44). Intravenous inoculation of pregnant WT SJL mice with a

Brazilian ZIKV strain caused intrauterine growth restriction of developing fetuses, cortical malformations, and ocular defects (45). However, SJL mice are not fully immunocompetent and express lower levels of IFN-stimulated genes than C57BL/6, and this difference may account for the varied susceptibility to the ZIKV (43). It has been demonstrated that the ZIKV NS5 protein inhibits type I IFN response in a species-specific fashion, which might explain why adult WT mice are more resistant to the infection (46,47). ZIKV infection has also been evaluated in immunocompetent neonatal mice (41,48,49). ZIKV inoculation in one-day old immunocompetent C57BL/6 or Swiss mice via subcutaneous or intracranial route resulted in neurological disease characterized by tremors, ataxia, and seizures with evidence of ZIKV infection in the brain (48,49). Since ZIKV is not naturally adapted to replicate in immunocompetent mice, their use in ZIKV research is limited. Nevertheless, immunocompetent mice have been used to assess the immunogenicity of vaccine candidates as well as their protective efficacy against viremia (34,43).

Immunocompromised mouse models

In order to develop a mouse model that can support ZIKV replication and disease, several groups have evaluated the ZIKV infection in immunocompromised adult mice. Several of these models have altered IFN responses, which are an important component of antiviral defense. Type I IFNs, which include multiple forms of IFN- α and one IFN- β , signal through the same receptor, termed *IFNAR1*, and have been commonly associated with innate immune responses to viruses (50). Both the IFN- α/β receptor and downstream signaling molecules, including STAT-1, are critical for protection from viral infection as mice deficient in these factors do not mount effective anti-viral responses (50,51). IFN regulatory factor (IRF), in particular IRF3 and IRF7, are essential for regulating the type I IFN response following viral infections (51). Type II IFN, or IFN- γ , which signals through a distinct receptor, *IFNGR1*, is the canonical cytokine of adaptive Th1 immunity and is essential for immune responses to intracellular pathogens (52). Animals lacking the receptor for type I IFN including A129 mice (129S2 *Ifnar1*^{tm1Agt}) and *Ifnar1*^{-/-} C57BL/6 mice or mice deficient in transcription factors *IRF-3/5/7*^{-/-} are highly susceptible to both African and Asian-lineage ZIKV and sustain infection with high viral loads in the brain (41,42,53-55). These animals developed severe ZIKV disease including hind-limb weakness, paralysis and death after peripheral (subcutaneous, intra-peritoneal, and intravenous) inoculation of ZIKV. Severity of ZIKV infection in these immunocompromised mice is age dependent, as older mice (11 week-old) are less susceptible to infection than younger mice (3-5 week-old) (41,42). Mice lacking both the type I and type II IFN receptors (AG129, 129/Sv *Ifnar1*^{tm1Agt} *Ifngr1*^{tm1Agt}) demonstrated greater susceptibility and more severe disease following ZIKV infection than A129 mice (21,25,42,56). Analysis of the tissues from ZIKV-infected A129 and AG129 mice demonstrated that the highest viral loads were in testes and brain. The presence of virus in the testes is consistent with the reports of sexual transmission of ZIKV. In addition, *Stat2*^{-/-} mice are also highly susceptible to ZIKV infection. After peripheral ZIKV inoculation, *Stat2*^{-/-} mice display neurological symptoms and virus was detected in the central nervous system (CNS), gonads and other visceral organs (57). *Stat2*^{-/-} mice lack both type I and type III IFN signaling. Subcutaneous inoculation of pregnant *Ifnar1*^{-/-} C57BL/6 mice at gestation days 6.5 and 7.5 with an Asian ZIKV strain resulted in fetal death and reabsorption in most of the fetuses while those that survived the infection had intrauterine growth restriction and growth impairment (44). For these experiments, *Ifnar1*^{-/-} female mice were mated with WT sires resulting in fetuses that were heterozygous for *IFNAR1*. Thus, despite the fetuses having the ability to respond to type I IFN, severe outcomes still were observed, suggesting that a type I IFN response in the fetus is not sufficient to protect from ZIKV-induced injury (44). Together, these immunocompromised

mouse models have been used to demonstrate ZIKV ability to cause fetal abnormalities, deterioration of gonadal tissue and infection through sexual route (43,44,58-61). Therefore, these susceptible mouse models also have been used extensively to evaluate candidate therapies and vaccines for efficacy against ZIKV replication (24,43,62-65). While these immunodeficient mice are useful, they are inherently biased toward producing disease based on their genetic background. Furthermore, lethality is the main endpoint without assessing actual clinical disease.

In an attempt to produce infection models that do not rely upon knockout mice, several groups have explored the temporal blockade of type 1 IFN in immune intact mice using polyclonal and monoclonal antibodies targeting either type 1 IFNs directly or the *IFNAR1* receptor. The major advantage of this approach is that it allows immune responses to be elicited in immunologically competent mice with type 1 IFN blockade only induced at the time of infection. It has been demonstrated that adult immunocompetent C57BL/6 mice treated with anti-IFNAR1 antibodies (that suppress expression of type 1 IFN) before infection are highly susceptible to mouse-adapted African ZIKV-Dakar strain (58,66). These mice develop severe ZIKV-mediated disease accompanied by significant neuroinflammation and mortality. Similarly, fetuses from mice with prior exposure to a blocking antibody against anti-IFNAR1 before ZIKV infection also resulted in intrauterine growth restriction (44).

Guinea pig model

Initial experiments conducted in 1950s showed that guinea pigs inoculated via intracranial route with the African ZIKV strain MR 766 developed no signs of infection (37). These studies used the prototype MR 766 strain of ZIKV, which had undergone extensive passage in suckling mouse brains. Recently, it has been demonstrated that guinea pigs are susceptible to infection by a contemporary Asian strain of ZIKV (67). Upon subcutaneous inoculation with PRVABC59 strain of ZIKV, guinea pigs demonstrated clinical signs of infection characterized by fever, lethargy, hunched back, ruffled fur, and decrease in mobility. ZIKV was detected in the serum using qRT-PCR and plaque assay. ZIKV infection resulted in a dramatic increase in protein levels of multiple cytokines, chemokines and growth factors in the serum. ZIKV RNA was detected in the spleen and brain, with the highest viral load in the brain (67). The guinea pig is more physiologically and immunologically similar to humans than other small animals. Specifically, the guinea pig's reproductive physiology and estrous cycle are similar to humans. Also, placentation in the guinea pig occurs in a manner similar to that of humans, and both guinea pig and human placentas are classified as hemomonochorial (68). Guinea pigs have a long gestation period and pups are born with a mature CNS, which makes this species a promising subject for studies of *in utero* transfer of ZIKV and neurological manifestations in infants (69).

LARGE ANIMAL MODELS OF ZIKV INFECTION

Non-human primate (NHP) models

NHPs also are being used to study ZIKV pathogenesis. In contrast to mice, immunocompetent macaque monkeys are ideal to study ZIKV because of the similarity in gestation and fetal development as compared to humans. Rhesus macaques are susceptible to infection by both African and Asian-lineage ZIKV (43,70-73). ZIKV-infected rhesus macaques developed viremia that peaked 2 to 6 days after infection and became undetectable by day 10. ZIKV was also detected in various organs, urine, saliva, and cerebrospinal fluid of

some animals (70,71). Similar to rhesus macaques, ZIKV infection was detected in several tissues of cynomolgus macaques including the male reproductive tract, intestines, and the brain and spinal cord (74,75). Pregnant rhesus macaques infected with ZIKV developed viremia lasting 30 to 55 days (70,72). Subcutaneous inoculation of a pregnant pigtail macaque with an Asian-lineage ZIKV resulted in reduced growth of the fetal brain (76,77). Autopsy analysis from fetal brain demonstrated ZIKV infection and substantial pathology to the CNS. ZIKV was detected in the placenta, fetal brain and liver, and maternal brain, eyes, spleen, and liver (77). ZIKV infection induced T-cell responses and protected NHPs from ZIKV re-infection and from heterologous ZIKV infection (70,74). Therefore, NHP models have been utilized for testing protective efficacy of novel vaccines and therapeutics against ZIKV (35,73,78). The NHPs reproductive cycle is similar to the human reproductive cycle, which has its clear benefits in the study of disease outcomes in pregnant mothers infected with ZIKV, but this comes with the caveat that the NHP models will produce data at a much slower rate. NHP models are very expensive to maintain and require a great amount of space and time when compared to other animal models.

CONCLUSION

Both small and large animal models have been established to investigate ZIKV pathogenesis and to develop vaccine and therapeutic strategies. ZIKV does not cause infection and clinical disease in weaned immunocompetent mice. Immunocompromised mice including IFN dysregulated mice have successfully reproduced clinical disease or demonstrated lethality after ZIKV infection. Immunocompetent guinea pigs are susceptible to infection by a contemporary strain of ZIKV. Similarly, NHPs have been demonstrated to be susceptible to infection by ZIKV. Together, these animal models of ZIKV pathogenesis can be utilized to evaluate candidate therapies and vaccines against ZIKV infection. However, each of these models has limitations that must be considered in the experimental design and interpretation of results.

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