Small animal models of Zika virus

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Abstract

Zika virus (ZIKV) infection can result in serious consequences, including severe congenital manifestations, persistent infection in the testes and neurologic sequelae. After a pandemic emergence, the virus has spread to much of the new world and has been introduced to many countries outside of endemic areas as infected travelers return to their home countries. Rodent models have been important in gaining a better understanding of the wide range of disease etiologies associated with ZIKV infection and for the initial phase of developing countermeasures to prevent or treat viral infections. We discuss herein the advantages and disadvantages of small animal models that have been developed to replicate various aspects of disease associated with ZIKV infection.

Acute viral disease

Table 1. Comparison of the advantages and disadvantages of small animal species used to model Zika virus infection and disease.

	 Wild-type mice	 Ab-induced immune deficient	 IFN pathway KO	 $STAT2^{-/-}$ hamsters	 Guinea pigs
Advantages	-Readily available -Inexpensive -Normal immunity -Well characterized -Diverse immune profiles	-Can use WT mice -Induced immune deficiency as needed -Support virus replication	-Age dependent disease -Virus replication -Diverse disease manifestations -Lethality	-Intermediate sensitivity -Persistent infection of testes -Lower mortality rate	-Similar placentation to human -Naturally susceptible to infection -Readily available -Normal immunity
Disadvantages	-Naturally resilient -Low virus replication -Requires high virus inoculum -Difficult infection routes	-Ab expense/ availability -Reversion to WT and clearance of virus -Little overt disease	-Limited availability -May require in-house colony -Sensitive to other pathogens -Abnormally severe disease	-Lack of reagents -Not commercially available -Variable disease manifestation -Underlying polyoma virus	-Lack of reagents -Large size -Not well characterized -Expensive to house -Somewhat difficult to work with.

Table 2. Mouse models of acute ZIKV infection and disease.

Mouse strain	Age (wk)	Virus, dose, route	Pathological findings		Ref.
			Neurological	Other	
Porton	0, 4	MR766, titration, i.c.	No disease reported after intracerebral inoculation	LD_{50} of $10^{7.2}$ pfu in 1-2 d.o. and $10^{6.4}$ pfu in adult mice	[1]
$IFNAR^{-/-}$or AG129	5, 11	Cambodian, 10^5 pfu, i.p.	Brain virus 3, tremors 6 dpi	Viremia 2 dpi, virus in spleen and testis	[2]
AG129	3-4 or 8	H/PF/2013, 10^{0-5} pfu, s.c., $10^{5.3}$ pfu, i.p.	Increased virus in brain, N	Viremia 2 dpi, myofiber necrosis, inflammatory cell infiltration, nuclear rowing	[3]
AG129	8	MR766, 10^{1-5} pfu, i.p.	P, C, N, viral Ag in brain and spinal cord, acute encephalitis	IFN-γ and IL-18 increased in serum, viral RNA in brain, spleen, liver, kidney	[4]
$IFNAR^{-/-}$	5-6	MP1751, 10^6 pfu, s.c.	PC, PMN cells in grey/ white matter near blood vessels, N in hippocampus.	Viral RNA in Br and Sp, 3 dpi, apoptosis in spleen, hematopoiesis in liver	[5]
AG129	8-10	P 6-740, $10^{3.0}$ pfu, s.c	P, C, Hyper-excitability, seizure, tremor. ZIKV Ag in neurons, astrocytes. Encephalitis and myelitis.	Viremia peak 5 dpi, virus in testis peaks 7 dpi, including infected Leydig cells, viral RNA shedding in urine	[6]
$IFNAR^{-/-}$ or *Irf3*$^{-/-}$ *Irf5*$^{-/-}$*Ifr7*$^{-/-}$	5-6	H/PF/2013 or MR766, 10^2 ffu, s.c. (f.p.)	ZIKV RNA in brain and spinal cord, higher paralysis after i.v.	Viremia 2-6 dpi, 10^6 ffu virus in testes of $IFNAR^{-/-}$	[7]
C57BL/6 + *Ifnar* Ab	5	DAKAR, $10^{6.4}$ pfu, s.c. or i.p.	Paralysis 8 dpi after i.p. challenge, viral RNA by ISH, ND in cerebellum and hippocampus	Viral RNA in brain, kidney, skeletal muscle & spleen 3 dpi, GFAP increase in cerebrum, brain titer 15 dpi	[8]

C57BL/6, IFNAR$^{-/-}$	0-1	PRVABC-59, $10^{3.3-4.3}$ pfu, s.c.	Wide array of neurologic symptoms, virus in neurons	Inflammatory gene increase, T cell driven immunity, viral load in brain and spleen	[9]
STAT2$^{-/-}$, IFNAR$^{-/-}$	5-6	MR-766, 10^3 pfu, s.c.	Viral RNA in brain, hind limb paralysis, cytokine elevations in the brain	Viral RNA in various tissues, upregulated IFN	[10]
BALB/c + Dexameth	6-8	PRVABC-59, $10^{6.5}$ pfu, i.p.	Neurons in hippocampus stain positive for ZIKV NS1	Orchitis	[11]
Swiss	0	SPH 2015, i.c. or s.c., NP	Neurological disease and death of neurons, gliosis	Atonic urinary bladder	[12]
STAT2$^{-/-}$	5-8	PRVABC-59, $10^{3.7}$ pfu, i.d.	Elevated ZIKV replication in spinal cord	Enhanced disease after convalescent sera Tx	[13]

Abbreviations: pfu - plaque forming unit; i.p. – intra peritoneal; s.c.- sub-cutaneous; i.d. – intradermal; TKO – triple knock-out; ffu- focus forming unit; P – paralysis; C – conjunctivitis; N – Neurodegeneration; PC – Perivascular cuffing; PMN – polymorphonuclear cells; NP- not provided.

There are many advantages to modeling viral disease in mice and other small animal species. Various advantages and disadvantages of rodent species that have been used to replicate disease manifestations of Zika virus (ZIKV) disease are included in Table 1. This paper will summarize the major findings of work performed in various small animal models to characterize disease outcomes after challenge with ZIKV.

Initial studies investigating ZIKV shortly after its discovery in the mid 1900s included challenge of various mouse strains, as well as a human challenge model [14-17]. These early studies demonstrated that mice were somewhat refractory to ZIKV infection [1], so work during the 20th century was limited to around 40 published reports. Emergence of ZIKV in the early 21st century resulted in an increased effort to learn more about this virus, with around 2,300 papers being published. One of the main goals was to develop small animal models for use in delineating the infection cycle, identifying consequences of virus infection and discovering antiviral countermeasures. A brief summary of the work to develop mouse models of acute ZIKV infection are included in Table 2.

More recent experiments were performed using immune compromised mouse strains, which were permissive for virus infection and displayed various neurologic signs of disease, including some that replicated severe disease manifestations in humans. The AG129 mouse strain, which lacks receptors for IFN-α/β and IFN-γ were susceptible to infection after inoculation with various strains of ZIKV injected by various routes [2-4, 6]. Virus replicates in a wide-range of tissues and viremia can be detected during the course of infection, with timing depending on the strain and route of virus. Relevant disease signs include eye lacrimation and neurologic involvement. The severity of disease observed in this mouse strain underscores the importance of the interferon response in controlling ZIKV infection.

Other interferon pathway knockout mice also show varying degrees of disease severity after infection with ZIKV and provide a suitable model for virus replication in tissues. Infection of IFNAR$^{-/-}$ mice, lacking IFN-α/β receptors, with ZIKV results in age-dependent severity, with mortality occurring in mice that are round 3 weeks or younger

[2]. The presence of IFN-γ receptors provides intermediate protection in 5-week old mice and complete survival in mice that are 11 weeks. Another knockout mouse strain, $IFNGR^{-/-}$ mice, lack IFN-γ receptors and are susceptible to ZIKV infection. These mice and were used to compare the pathogenicity of various ZIKV infectious clones [18]. Mosquito transmission of ZIKV to $IFNAR^{-/-}$ mice has helped identify the vector competency for several virus strains [19]. $STAT2^{-/-}$ knockout mice and mice lacking interferon response factors (Irf) 3, 5 and 7 were also sensitive to intravenous (i.v.) infection with ZIKV [7, 10].

Histologic analysis identified ZIKV-positive neurons found throughout the central nervous system, including neurodegenerative multifocal neutrophilic encephalitis and myelitis. The observation of ZIKV in motor neurons in the ventral horn of the spinal cord was similar to disease observed after infection with West Nile virus [20]. Astrocytes were also heavily infected in various regions of the brain and spinal cord after ZIKV infection, which was similar to pathology observed after challenge of mice with Venezuelan equine encephalitis virus, an alphavirus [21]. Interestingly, infected AG129 mice displayed rear limb myofiber degeneration and necrosis with inflammatory cell infiltration in the absence of hind-limb paralysis, suggesting that this virus infects muscle cells [3]. Direct intraocular inoculation with ZIKV results in infection of the cells lining the blood-retinal barrier and causes chorioretinal atrophy [22].

Since wild-type mice generally display only a very transient viral infection after ZIKV challenge, various methods are used to increase the susceptibility of wild-type, immunocompetent strains. Mice can be treated with function-blocking antibodies (MAR1-5A3) targeting the IFN-α/β receptors to increase the susceptibility of the mice just prior to challenge with virus [8]. Viral RNA was detected in the cerebrum and hippocampus in mice treated with function-blocking antibody. Dexamethasone immunosuppression renders BALB/c mice susceptible to ZIKV infection, resulting in detectable virus titer in various tissues and resulting in lethality [11]. Infection of neonatal mice shortly after birth also results in morbidity and mortality with more severe disease and death observed in the younger mouse pups [12]. Infected neonates developed neurologic complications, including tremors, seizure, hyperactivity and limb collapse with detectable virus in the brain as late as 15 dpi.

Preexisting flavivirus antibodies (Ab) are implicated in worsened disease during infection, a concept known as antibody enhancement of disease. This phenomenon is well-known for dengue virus (DENV), where Ab to one strain binds at low levels to an incoming heterotypic strain, resulting in an increased uptake of virus by Fc-receptor-bearing cells of the immune system. Infection in this manner effectively increases the host cells available for viral replication and thereby increases the antigen load, host response and immunopathology of the infection [23]. This has been demonstrated in mouse models of DENV infection [24, 25]. Because of the overlap in sequence between DENV and ZIKV, it was also anticipated that antibody enhancement of disease might also play a role in ZIKV pathogenesis. although there is no clinical evidence to support this idea. Enhanced disease in ZIKV infected mice after treatment with immune serum containing Ab to DENV or West Nile virus (WNV) has been demonstrated [13].

Enhancement of disease should be carefully considered in the design and implementation of vaccines.

Aside from mouse models, other species have been used to model ZIKV, including chickens and guinea pigs. Infection of chicken embryos with ZIKV results in virus infection of the developing nervous system, causing fetal demise at higher virus challenge doses and a microcephaly-like phenotype at lower doses, replicating some aspects of congenital infection [26]. Immunocompetent guinea pigs infected with ZIKV developed a transient viremia, increase in cytokines and chemokines, infection of various tissues and development of neutralizing Abs [27]. These additional models of ZIKV infection and disease may provide useful systems for the evaluation of countermeasures or in disease characterization.

Congenital infection

Table 3. Rodent models of congenital infection with ZIKV.

Strain, Rodent	Origin, Virus strain	Infection route/dose	Day of gestation	Major findings	Ref.
IFNAR1$^{-/-}$ X C57BL/6 mouse	French Polynesia, H/PF/2013	s.c., f.p./10^3 ffu	E6.5, 7.5	Fetal and placental infection, IUGR, fetal demise, placental and fetal brain apoptosis	[28]
C57BL/6 mouse	French Polynesia, H/PF/2013	(MAR1-5A3 Ab E5.5) s.c., f.p./10^3 ffu	E6.5, 7.5	Placental and fetal infection, IUGR	[28]
SJL mouse	Brazil Paraiba 2015	i.v./10^3/$10^{10.6}$, 10^{12} pfu/ml	E10-13	IUGR, upregulation of apoptosis genes, cortical malformations	[29]
ICR mouse	SZ01	l.v. inj. of fetus/$10^{5.8}$ pfu/ml (1 µl)	E13.5	Brain replication in VZ and SVZ, cortical thinning, infection of NPCs or IPCs	[30]
C57BL/6 mouse	SZ01	i.u. Fetal Brain Inj/$10^{5.5}$ pfu	E13.5	ZIKV infection of placenta and fetal brain, reduction of cortex founder cells	[31]
C57BL/6, IRF3/7$^{-/-}$, IFNAR$^{-/-}$ mouse	Cambodian FSS13025	ivag/$10^{4.4}$-$10^{5.7}$ pfu	E4.5-8.5	IUGR, fetal demise, ZIKV infection of fetal brain (RNA and EM)	[32]
CD-1 mouse	2010 Cambodia 2015 Brazil 2015 Puerto Rico 1968 Nigerian	i.u., i.p./10^6 $TCID_{50}$	E10, 14	High aborted fetus rate (30-45%), virus in fetus/dam and IFN 48 hpi w/infection at E10 (not at E14). Neuroinflam. and cortical thinning in neonatal brain	[33]
STAT2-/- hamster	Malaysia, P 6-740	s.c./$10^{2.7}$ $CCID_{50}$	E8.5	Virus in fetal brain, placental pathology, live births	[34]
FVB/NJ C57BL/6	Bahia, Brazil, HS-2015-BA-01	i.v. (jugular)/ 10^5 PFU	E5.5, 7.5, 9.5	Infection of placenta/fetus, pathology of fetal brain, fetal demise, arthrogryposis	[35]

Abbreviations: s.c.- sub-cutaneous; f.p.- footpad; E- embryonic day; IUGR- intrauterine growth restriction; ffu- focus forming units; i.v.- intravenous; pfu - plaque forming unit; l.v.- lateral ventricle (fetus); VZ-

ventricular zone; SVZ- subventricular zone; NPC- neural progenitor cells; IPC- intermediate progenitor cells; i.u.- intrauterine; inj.- injection; ivag- intravaginal; EM- electron microscopy; i.p.- intraperitoneal; TCID50- 50% tissue culture infectious dose; IFN- interferon (type I); CCID50- 50% cell culture infectious dose;

Intrauterine exposure of a developing fetus to ZIKV infection can result in debilitating manifestations in the fetus, the most severe and obvious of which is microcephaly. An increase in the incidence of microcephaly during the recent ZIKV outbreak in Brazil eventually led to the discovery of the causative role of the virus in developmental abnormalities of fetuses after exposure *in utero*. Aside from microcephaly, other disease manifestations have been reported, including smaller birthweight, brain abnormalities despite normal head size, hearing loss, optic nerve hypoplasia, joint and bone deformities (arthrogryposis), and many other less apparent effects of ZIKV congenital exposure [36]. A significantly ($P<0.0001$) higher mortality rate (~5.1%) of fetuses was associated with ZIKV infection cases as compared with other etiologies (~1.4%) in pregnant women in Brazil [37]. This article will focus on various consequences of intrauterine exposure to ZIKV that have been replicated in small animal models as well as those aspects of congenital infection that are dissimilar between rodents and humans.

In attempting to recapitulate disease associated with intrauterine infection in small animal models, various laboratories have independently developed mouse models of congenital infection (Table 3). Despite the use of a diverse range of ZIKV strains, as well as differences in dose, route and timing of infection during gestation, some consistent consequences of infection have been delineated in rodents. Virus was generally detected in the placenta [28, 31, 34, 35], while fetal infection was dependent on timing of maternal challenge and typically correlated with virus titer in the placenta. A gestational time-dependent transmission was also observed, with lower fetal infection rates at later stages of gestation. Another commonly observed consequence was intrauterine growth restriction (IUGR) and spontaneous abortion of developing fetuses [28, 29, 32]. These findings are similar to outcomes in natural congenital infection and can be used to further characterize congenital infection as well as to identify countermeasures to inhibit or prevent fetal infection. The period between embryonic day (E)3 and E14, when pregnant mice have been challenged with ZIKV across several studies, corresponds with days 4-48 of human gestation during the first trimester.

The interaction between virus in maternal blood and fetal tissues occurs in the placenta. Although the placental structure of mice is quite distinct from that of humans, there are various similarities that are found between the two that make the mouse a reasonable model for various aspects of human placentation, including notably the lining of fetal structures with syncytiotrophoblast cells that contact maternal blood [38]. Trophoblast cells of the mouse conceptus will differentiate, forming a branched villious structure, which invades the placental wall decidua beginning around E8.5. The placenta of mice is considered functional at approximately 10 days post-coitus (dpc) [39-41].

The timing of virus challenge during pregnancy is important in the context of congenital infection of the fetus in rodent models. Congenital infection of mice with other flaviviruses was demonstrated when virus challenge occurred between E5 and E12, but detection of virus or viral antigen in the fetus was less likely as development proceeded past E12 [42, 43]. Similar observations have been reported with ZIKV infection in mouse models [33, 35]. Although peripheral challenge of pregnant dams with ZIKV after E12 results in virus infection of the placenta, infection at this time does not typically result in fetal infection in rodents. The timing of reduced congenital virus infection in rodents appears to be associated with placental development. This is somewhat dissimilar to congenital infection in humans, where ZIKV infection during the second and third trimester can result in transmission to the fetus. This observation could indicate partial control of the dissemination of virus to the fetus at the placental barrier, or could suggest other mechanisms involved with transplacental infection.

A functional placenta is present in mice around E10. Challenge of mice between E4 and E14 results in productive placental infection in mice. The detection of virus in the placenta after challenge from E4 to E14 sugests a high degree of susceptibility of this organ [33]. After the development of a functional placenta in mice, transplacental transmission of ZIKV and other flaviviruses to the fetus is reduced, unless the fetuses are challenged directly [30, 31, 33]. The lack of infection at later times in mice may suggest the placental barrier is preventing transplacental movement of ZIKV in the mouse. However, this is dissimilar to congenital infection of human fetuses, where brain lesions have been observed in newborn babies after ZIKV infection during the second and third trimester of pregnancy [44]. However, these second and third trimester infections of human placentas appear to be limited primarily to Hofbauer cells (HBCs) and the inflammatory villitis that is seen during first trimester placental infections is reportedly absent at later infection times [45]. Indeed, HBCs isolated from human placentas have been the focus of several studies, which demonstrate susceptibility of these cells to ZIKV [45-47]. In contrast, infection of the placenta during the first trimester of human pregnancy results in the infection in a wider array of placental cells. Mesenchymal cells and cytotrophoblasts support ZIKV replication along with HBCs. This gestationally-dependent infection of placental cells was further supported by a study that demonstrated expression of genes for various entry factors utilized by ZIKV in early-stage type trophoblast cells [48]. These cells were highly susceptible to infection in cell culture, while cells isolated from term placentas did not express these genes and were relatively resilient to ZIKV infection. While mice and humans may differ in some aspects of congenital infection, challenge of pregnant mice during early (prior to E10) gestation models many aspects of natural infection.

Most of the studies have terminated at some point during gestation and few live births have been recorded. The consequences of intrauterine exposure to ZIKV on the development of neonates will be an important step in future research to further identify consequences of congenital infection. Depending on the strain of virus used, the immune state of the dam, and the timing of infection, the aborted fetus rate can be relatively high [33], which would result in fewer births. Disease manifestations in females just after birth may cause the dams to neglect or cannibalize pups [34], further

complicating studies designed to characterize the effect of intrauterine exposure to ZIKV on the development of neonates. Future studies should include live births to determine the suitability of mice to model the effects of intrauterine infection on developing neonates.

Sexual transmission

Table 4.

Model	Virus origin, strain	Infection route/dose	Major findings	Reference
C57BL/6 (MAR1-5A3) Rag1$^{-/-}$ Axl$^{-/-}$ mice	Dakar 41519 (ma) H/PF/2013	s.c. (f.p.)/$10^{6.0}$ pfu $10^{3.0}$ pfu	Persistence of ZIKV in T/E (21 dpi), reduced testis size, low testosterone, infected spermatogonia, seminiferous tubule degradation	[49]
IFNAR$^{-/-}$ mice	MEX2-81	s.c. (f.p.)/10^5 pfu	Presence of viral RNA in T/E 21 dpi, T atrophy, reduced testosterone	[50]
IFNAR$^{-/-}$ mice	ZIKA-SMGC-1	i.p./$10^{3.0}$ pfu i.t./$10^{2.6}$ pfu	Atrophy of the repro tract, virus in T/E, not seminal vesicle or prostate	[51]
AG129 mice	Puerto Rico, PRVABC59	i.p./10^3 pfu	ZIKV RNA/virus observed in semen, demonstration of sexual transmission of virus and fetal infection, virus/inflammation in T/E	[52]
STAT2-/- hamster	P 6-740	s.c./$10^{2.7}$ $CCID_{50}$	Infection of Sertoli cells and spermatogonia	[34]
BALB/c mice (dexameth.)	Puerto Rico, PRVABC59	i.p./$10^{6.5}$ pfu	Pathology of seminiferous tubules, lymphocytic infiltration (12-14 dpi), reduced viral load in T/E and prostate after IFN treatment	[53]
AG129 and LysMCre^{+}IFNAR	Cambodia, FSS13025	Ivag/ 10^5 or 10^6 ffu	Estrus cycle-dependent susceptibility of females	[54]
IFNAR$^{-/-}$ mice	Puerto Rico, PRVABC59	Rectal/$10^{6.5}$ pfu	Nonlethal, virus in rectum, testis, brain and spleen d21, inflammation and splenomegaly	Martinez et al., 2017

Abbreviations: s.c.- sub-cutaneous; f.p.- footpad; pfu- plaque forming units; T- testicle; E- epididymis; i.p.- intraperitoneal; i.t.- intratesticular; s.c.- sub-cutaneous; dpi- days post-virus injection; Ivag- intravaginal; ffu- focus forming units;

A somewhat unanticipated consequence of ZIKV infection is sexual transmission. Evidence of non-mosquito transmission was available as early as 2008, where a returning scientist that was infected with ZIKV in Senegal transmitted the virus to his wife, which was suspected to be through sexual contact [55]. Closely related flaviviruses, such as dengue virus, are not known to be transmitted through sexual contact. It has been well documented that men that were exposed to ZIKV in endemic areas could further spread the virus to their sexual partners, which may or may not include the manifestation of disease. Various clinical studies have provided evidence of sexual transmission of ZIKV from male-to-female, female-to-male and male-to-male [56-60]. Transmission has been reported to occur several weeks after returning from areas of endemicity and virus (typically viral RNA) could be recovered from the semen of infected males up to 6 months after disease onset [61]. While little is known about the localization of virus in the reproductive tract of infected men, mouse models have

provided insight into which cell types are infected and potential mechanisms of sexual transmission (Table 4).

Many published reports have demonstrated ZIKV infection of the male reproductive tract of rodents, primarily indicating detection of infectious virus or viral RNA in the testes of infected males [2, 4, 6, 7]. The testes support very high levels of virus, particularly in immunocompromised mice. Virus has been localized in various cells of the testis, including Leydig cells, spermatogenic precursors, and in epithelial cells of the epididymis [34, 52]. Interstitial inflammation and inflammatory cell infiltrates were observed. Necrotic cells that stained positively for viral antigen were observed in the lumen of the epididymides [49], representing a potential source of virus for transmission. Other severe disease manifestions, such as orchitis and testicular atrophy, have been observed in mouse models [50].

The implications of testis infection observed in mice may not be directly translatable to human infection. For example, orchitis, or inflammation of the testicle, is a very painful condition in people and is generally the consequence of bacterial infection, including sexually transmitted infections. This is commonly observed in immune suppressed mice infected with ZIKV [11, 51]. If a common consequence of ZIKV in infected men was orchitis, the pain would be intense and they would surely seek medical attention. Orchitis is likely not a commonly occurring consequence of ZIKV infection of the male reproductive tract.

Intravaginal infection of female mice with ZIKV can cause a systemic infection and may also be transmitted to offspring [52, 54]. The estrous cycle may influence the susceptibility of females, as AG129 mice that were inoculated intravaginally with ZIKV during a hormonally-induced estrus-like phase did not succumb to viral infection and had a relative lack of virus replication in various tissues, while those that were in an induced diestrus-like phase displayed virus replication and mortality [54]. Transmission from infected males to naïve females has been demonstrated in AG129 mice, including transmission from vasectomized mice, despite a lower virus load as compared with intact males [52]. Males also had detectable virus in the semen several weeks after virus challenge, modeling persistent virus present in men infected naturally with ZIKV. Rectal inoculation of male mice results in systemic spread of virus to various tissues, including the testes and supports observations of male-to-male transmission (Martinez, 2017 preprint).

Use of models in the development of countermeasures

Table 5. Various anti-ZIKV countermeasures have been tested in small animal models.

	Strain, age	Virus strain	Treatment protocol	Outcome	Ref
Antivirals	AG129, 8-14 wks	MR766	7 DMA, p.o., 50 mg/kg/d, qd X 10 beginning -1 hour	Reduced viremia by 0.5-1.3 $\log_{10}$, delayed MDD 15-23 dpi	[4]
	AG129, 8-10 wks	P 6-740	BCX4430, 300 mg/kg/d, i.m., bid X 7 beginning 0-7 dpi	Reduced viremia on 5 dpi by ~2 $\log_{10}$, delayed or prevented death, reduced disease, survive re-challenge	[6]

	IFNAR$^{-/-}$, 4 wk	GZ01/2016	NITD008, 50mg/kg, p.o at 4,24,48,72 and 96 hpi	Reduced viremia 2.6-fold on 2dpi, 50% survival, reduced disease signs	[62]
	C57BL/6 + *Ifnar1* mAb	DAKAR 41519	Sofosbuvir, 33 mg/kg/d, oral X 7, beg. 24 hpi	Improved survival, extended MDD	[63]
	BALB/c, 6-8 wks + DEX	PRVABC-59	Peg. IFN-α2b, $10^{3.3}$ IU, s.c., q 96 h, 1-9 dpi	Reduced viral loads in various tissues, 100% survival, no prominent inflammation in any of the tissues tested	[11]
			IFN-β1b, $10^{5.2}$ IU/dose, i.p., q 48 h, 1-9 dpi		
Antibody Therapies	Prenant ICR mice E13.5	SZ01	Convalescent sera, 100 µL, i.p. E14.5 (1 dpi) and E15.5 (2 dpi).	Neutralizing Ab titer of 161, reduced caspase-3 in cortex, rescued cortical plate thinning	[64]
	IFNAR$^{-/-}$	H/PF/2013	Human mAb from DENV patients, 10µg, i.p., -1 and 9 dpi	mAbs EDE1-C10 neutralized ZIKV, 100% survival	[65]
	C57BL/6 + *Ifnar1* mAb	ZIKV- Dakar	mAb ZIKV-117, from human antisera, i.p., 6.7 mg/kg 1 dpi or 16.7 mg/kg, 5 dpi	100% survival (1 dpi) or 70-75% (5 dpi). Reduced transmission, pathology and mortality	[66]
	Ifnar1$^{-/-}$ female X WT male	ZIKV- Dakar (E6.5)	mAb ZIKV-117, dams treated with 6.7 mg/kg, 1 dpi or 16.7 mg/kg, 5 dpi	4-5 $\log_{10}$ reduced virus in fetal and maternal tissues, protection in pregnancy model due to neutralization	
	Pregnant C57BL/6 mice	ZIKV- Dakar (E5.5)	mAb ZIKV-117, dams treated with 16.7 mg/kg 1 dpi	Reduced virus in dams, placenta and fetus, improved fetal, placental disease, prevents vertical transmission	
Vaccine	AG129, 4-6 wks	FSS13025 or MR 766	Inactivated MR 766 virus, vaccine 10 µg / dose, 0 and 21 days, i.m.	100% survival in MR 766 & FSS13025 infection, 100% survival, absence of detectable viremia in serum up to 6 dpi.	[67]
	BALB/c, C57BL/6 or SJL, 4 wk	ZIKV2015, PRVABC-59	DNA-prM-Env or DNA-Env vaccines, 50 µg, i.m, -28 dpi	Reduced viremia, correlation of efficacy w/ neutAb, Ab transfer improves	[68]
	IFNAR$^{-/-}$	FSS13025	10-del ZIKV, route, -4 wks	High neutAb, robust T cell response, 100% survival, undetectable viremia.	[69]
	CD-1, 1 day old	FSS13025	10-del ZIKV, as above	100% survival	
	AG129, 6 wks	P6-740	prM-E mRNA, -6 wks	High neutAb, 100% survival	[70]
	C57BL/6 + *Ifnar1* mAb, 8 or 18 wk	Dakar 41519	modified prM-E mRNA, -6 wks	High neutAb, 100% survival	
	C57BL/6, BALB/c	MR-766	VSV-ZprME, VSV-ZENV, i.v. or i.m., -3 wks	Offspring born to vaccinated females were protected from challenge on PND7	[71]

Abbreviations: wks – weeks; p.o- per os; qd- once a day; dpi- days post-virus injection; i.m. – intramuscular; bid- twice a day; i.p – intraperitoneal; hpi- hours post-virus injection; pfu - plaque forming unit; MDD - mean day-to-death; neutAb- neutralizing antibody; s.c.- subcutaneous; i.d. – intradermal; i.c – intracerebral; RDRP – RNA dependent RNA polymerase; i.v – intravenous; ffu – focus forming unit; conv-convlescent serum; PND- post-natal day.

As there are currently no FDA-approved drugs to treat acute flaviviral diseases, it is unknown how direct-acting antiviral compounds would impact infection and disease outcome in people infected with ZIKV. There is potential for antiviral treatment to reduce disease burden and further spread of the virus if therapy is initiated soon after the onset of clinical disease or if prophylactic treatment, in the context of a wide-spread viral outbreak, is used. In regards to the treatment of Zika, antiviral agents could have use in preventing fetal transmission, clearing sequelae from the testes or reducing viral load during acute infection to reduce or inhibit further transmission.

Various nucleoside analogs, including 7-deaza-2'-C-Methyladenosine (7DMA), BCX4430, sofosbuvir and NITD008 have been shown to be active against ZIKV in cell culture and in mouse models [4, 6, 62, 63]. These viral RNA-dependent RNA polymerase (RdRp) inhibitors delay or prevent mortality, reduce virus titer in relevant tissues, and improve disease in infected mice. Although these broad-spectrum antivirals did not completely eliminate disease, efficacy in knockout mouse models was nevertheless impressive due to the acute sensitivity of these mice to ZIKV and would likely fare better against natural infection in an immunocompetent host. Sofosbuvir is an FDA-approved drug used to treat chronic hepatitis C virus (HCV) [72]. If this compound has clinical efficacy against ZIKV, the approval process would be truncated as the compound is well-characterized for human administration. BCX4430 has been shown to be effective against a wide range of viruses of human concern [73-75]and clinical trials have been initiated. NITD008 was initially identified as a potential antiviral to treat DENV, but had toxicity after long-term treatment [76]. Short-term treatment, applicable to treatment of acute arboviral diseases, did not result in appreciable toxicity and further clinical investigation may be warranted. Broad-spectrum activity of NITD008 has also been observed in various animal models [76, 77].

Indirect acting antiviral agents have also been identified to have activity against ZIKV in mouse models. The compound 25 hydroxy cholesterol (25HC) is an enzymatic product of cholesterol-25-hydroxylase. Treatment with 25HC reduced ZIKV viremia by blocking viral entry in mice and macaques, including reduction of microcephaly in a congenital model [75]. Azithromycin, a macrolide antibiotic that is FDA-approved for use including during pregnancy, prevented ZIKV production and viral mediated cell death in primary human brain tissue by reducing viral proliferation and cytopathic effects in glial cells and astrocytes [78]. However, activity has not yet been demonstrated in a small animal model.

Antibody (Ab) therapy has been used to control virus infection and disease during outbreaks [79]. Antibodies targeting the envelope (E) protein, and in particular domain III, have been shown to include potent neutralizers that would be suitable for use in therapy [80, 81]. Efficient neutralizing Abs have been isolated from the serum of people that have been infected with ZIKV, revealing the importance of antibodies targeting the envelope protein (E) in the context of clearance of ZIKV infection [66]. Some of these antibodies have shown activity in mouse models, and are effective in preventing or reducing disease after ZIKV infection, including prevention of congenital infection after treatment of pregnant dams [64, 66]. This is consistent with previous studies with the

related WNV, where prevention of congenital infection was observed in rodent models treated with pooled human immune serum [82].

Care must be taken in regard to use of Ab therapy in the context of ZIKV, as treatment with convalescent plasma from donors possessing Abs specific for flaviviruses, including DEN and WNV, may enhance disease [13]. Antibodies containing mutations in the Fc receptor region maintain the specificity and neutralizing capabilities of the Ab, but remove the possibility for negative enhancement interactions of Ab with immune cells. Antibodies with inactivated Fc receptor have been shown to be effective in small animal models [66].

Vaccines are very important in controlling acute arboviral diseases and have demonstrated efficacy in substantially reducing the disease burden of yellow fever virus (YFV), the archetypical flavivirus [83]. Various types of vaccines have been developed to protect against ZIKV (Table 5) and additional studies are being published at a rapid pace. Inactivated or attenuated viruses may also elicit long-term immunity as is the case with the attenuated 17D YFV vaccine. Inactivated and modified live-attenuated (10 nucleotide deletion in in the 3'-UTR) viruses have shown promise in preventing ZIKV in mouse models [67, 69]. Development of vaccines that target the E protein have been prominent and show promise in various animal models [68, 70]. As with Ab therapy, an important question in regard to vaccine development is whether the Abs elicited by vaccination might serve to enhance infection with ZIKV. Some reports of enhancement in small animal models have been reported [13].

Conclusions

Small animal models are important in delineating the consequences of ZIKV. The use of various rodent species and strains, including genetic knockouts, has provided useful information to help us better understand disease as a result of infection with this virus. Continued efforts will provide information to aid in the development of countermeasures to reduce the disease burden of this emerging virus.

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