Zika virus pathogenesis and tissue tropism

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Abstract

Although Zika virus (ZIKV) was isolated approximately 70 years ago, few experimental studies had been published prior to 2016. The recent spread of ZIKV to countries in the Western Hemisphere is associated with reports of microcephaly, congenital malformations and Guillain-Barré syndrome. This has resulted in ZIKV being declared a public health emergency and has greatly accelerated the pace of ZIKV research and discovery. Within a short time period, useful mouse and non-human primate disease models have been established, and pre-clinical evaluation of therapeutics and vaccines has begun. Unexpectedly, ZIKV exhibits a broad tropism and persistence in body tissues and fluids, which contributes to the clinical manifestations and epidemiology that have been observed during the current epidemic. In this review, we highlight recent advances in our understanding of ZIKV pathogenesis, tissue tropism and the resulting pathology, and discuss areas for future investigation.

INTRODUCTION

Zika virus (ZIKV) is a mosquito-transmitted Flavivirus in the Flaviviridae family of positive-stranded RNA viruses, and is closely related to several other pathogens that cause disease globally including Dengue (DENV), yellow fever (YFV), West Nile (WNV), Japanese encephalitis (JEV), and tick-borne encephalitis (TBEV) viruses (Pierson and Diamond, 2013). Similar to DENV and YFV, ZIKV can circulate directly between Aedes mosquitos and humans, and thus is capable of epidemic transmission (Vasilakis and Weaver, 2017). Although ZIKV was first isolated in 1947 from a febrile rhesus macaque at the Zika

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Forest Research Station in Uganda (Dick et al., 1952), many of its distinguishing features have been discovered only recently after the virus spread globally and the epidemic force of infection increased. The ZIKV genome consists of an ~11 kb positive-stranded RNA molecule that encodes 3 structural and 7 non-structural proteins. The virion has a diameter of ~50 nm and contains a nucleocapsid that is surrounded by a lipid bilayer containing the structural proteins (prM/M and E), which are arranged with icosahedral symmetry on the surface (Kostyuchenko et al., 2016; Sirohi et al., 2016; Vasilakis and Weaver, 2017).

**Clinical features of ZIKV infection**

Historically, the clinical syndrome caused by ZIKV in humans was mild, consisting of a self-limiting flu-like febrile illness that resolved within days and occurred in an estimated ~20% of infected individuals (Duffy et al., 2009; Simpson, 1964). During the recent epidemic however, ZIKV infection has been associated with severe disease, including multi-organ failure (Swaminathan et al., 2016) and thrombocytopenia and thrombocytopenic purpura (Karimi et al., 2016). In comparison to the encephalitic flaviviruses (e.g., WNV and TBEV), ZIKV generally is less neuroinvasive in adults, and rarely causes meningitis and encephalitis (Carteaux et al., 2016). However, ZIKV preferentially infects and injures neural progenitor cells (Tang et al., 2016), which may explain its ability to impair development of the fetal brain and cause microcephaly and other neurodevelopmental injuries. ZIKV also can infect the eye and cause uveitis in adults, a potentially blinding inflammatory disease (Furtado et al., 2016; Miner et al., 2016b). ZIKV infection results in conjunctivitis in up to 15% of patients, perhaps due to direct infection of the eye (Duffy et al., 2009; Miner et al., 2016b; Sun et al., 2016).

The most alarming feature of ZIKV infection during the recent North American outbreak has been its ability to cause microcephaly, congenital malformations, and fetal demise. In a case series of symptomatic, ZIKV-infected pregnant women in Brazil, a remarkable 29% of fetuses exhibited some type of ultrasound abnormality (Brasil et al., 2016a; Brasil et al., 2016b). The clinical phenotype of congenital ZIKV infection was variable, and included cerebral calcifications, microcephaly, intrauterine growth restriction, and/or fetal demise. Retrospective assessment of the ZIKV epidemic in French Polynesia also found an increased risk of microcephaly associated with ZIKV infection, with 95 cases occurring per 10,000 women infected in the first trimester (Cauchemez et al., 2016). Computed tomography and magnetic resonance imaging of the brains of congenitally infected neonates in Brazil have demonstrated hypoplasia of the cerebellum and brainstem, ventriculomegaly, delayed myelination, enlarged cisterna magna, abnormalities of the corpus callosum, calcifications, and cortical malformations (de Fatima Vasco Aragao et al., 2016). Congenital ZIKV infection can also result in sensorineural hearing loss and blindness in humans with a variety of abnormalities of the eye including retinal mottling, lens subluxation, and optic neuritis (de Paula Freitas et al., 2016; Leal et al., 2016).

ZIKV appears to induce or mimic autoimmunity in a limited number of cases by unknown mechanisms (Oehler E et al., 2014; Zea-Vera and Parra, 2016). During the ZIKV epidemic in French Polynesia, case reports were described of ZIKV-associated Guillain-Barré syndrome, which is characterized by ascending paralysis and polyneuropathy (Oehler E et
An association of ZIKV infection with Guillain-Barré syndrome also was reported in Brazil, Colombia, and other countries (do Rosário et al., 2016; dos Santos et al., 2016). Guillain-Barre syndrome may occur concurrently with acute ZIKV infection or in its immediate aftermath (do Rosário et al., 2016; Siu et al., 2016), suggesting that demyelination of peripheral nerves is due to direct infection and/or autoimmune-mediated targeting of neurons and glial cells. Electrophysiological studies demonstrated an acute axonal neuropathy and laboratory analysis revealed the presence of autoantibodies (e.g., anti-glycolipid and anti-GA1 antibodies), although the typical anti-ganglioside antibodies associated with Guillain-Barré syndrome were detected only rarely in these patients (Cao-Lormeau et al., 2016).

Human-to-human transmission

Unlike most other flaviviruses, a component of the spread of ZIKV may reflect its potential for human-to-human transmission. The first described case of sexual transmission involved an American, who contracted ZIKV infection while working in Senegal in 2008 and later transmitted it to his wife upon his return to the United States (Foy et al., 2011). Subsequently, multiple studies have reported suspected sexual transmission of ZIKV, and infectious virus can be detected in semen or sperm of infected males (Barzon et al., 2016; Mansuy et al., 2016). Female-to-male and male-to-female sexual transmissions of ZIKV also have been reported (Davidson et al., 2016; Musso et al., 2015). Modeling has suggested that sexual transmission contributes to approximately 3% of human cases (Gao et al., 2016), although this estimate has not yet been corroborated. In another human-to-human transmission case, ZIKV was acquired from a hospitalized patient via an unknown, non-sexual route through presumed contact with other infected body fluids (e.g., tears or saliva), which contain infectious virus as well as non-infectious viral nucleic acids (Dudley et al., 2016; Miner et al., 2016b; Sun et al., 2016; Swaminathan et al., 2016). Additionally, ZIKV has been transmitted by blood and platelet transfusion (Barjas-Castro et al., 2016; Motta et al., 2016). Although more study is warranted, the unique ability of ZIKV, relative to other flaviviruses, to spread between humans through non-insect vector routes may reflect one of several factors including its possible enhanced stability in body fluids or its broad cell and tissue tropism (Figure 1). A cryo-electron microscopy structure of ZIKV virion revealed a more compact structure than other flaviviruses, which may explain the greater stability of ZIKV at 40°C compared to DENV (Kostyuchenko et al., 2016). However, the greater physical stability of ZIKV was questioned in more detailed comparison studies with DENV and WNV (Goo et al., 2016). Further experiments are required to define how the relative stability of ZIKV in various body fluids affects its potential transmission.

ZIKV pathogenesis in humans and animal models

In humans, ZIKV antagonizes the type I interferon (IFN) response, in part through its NS5 protein, which promotes proteasomal degradation of STAT2 (Grant et al., 2016; Kumar et al., 2016) a transcription factor that is activated downstream of signaling by the type I IFN receptor (Ifnar1). In comparison, ZIKV NS5 did not bind to or promote degradation of mouse Stat2 (Grant et al., 2016), which in part, may explain why immunocompetent mice are not natural hosts for the virus. To develop models of ZIKV pathogenesis in mice, some
laboratories have inoculated immunocompetent animals intravenously with high titers of ZIKV (Cugola et al., 2016), which presumably bypasses innate immune responses in peripheral organs, whereas others have infected Ifnar1\(^{-/-}\) or Ifnar1\(^{-/-}\) Ifngr\(^{-/-}\) double knockout (AG129) mice via subcutaneous inoculation (Aliota et al., 2016a; Lazear et al., 2016; Rossi et al., 2016).

The first ZIKV strain (MR 766) isolated, in 1947 in Uganda, pre-dated the development of tissue culture, and was passaged serially in the brains of mice ~100 times (Dick et al., 1952). Passaging of virus in a specific organ can result in adaptation of the virus to a specific tissue or cell type, attenuation of the virus in other tissues or cell types, or accumulation of mutations such that the passaged virus drifts genetically from the original clinical isolates. Thus, studies using MR 766 and other highly passaged ZIKV should be validated with other more recent clinical strains whenever possible. Multiple clinical ZIKV strains from Africa, Asia, and the Americas have been isolated and serve as important tools for studies of ZIKV pathogenesis. Many strains have been sequenced, and some developed into infectious clones for analysis of the genetic determinants of ZIKV pathogenesis (Schwarz et al., 2016; Shan et al., 2016; Tsetsarkin et al., 2016; Weger-Lucarelli et al., 2016).

Since adult wild-type immunocompetent mice are resistant to lethal ZIKV infection, one approach to study pathogenesis has been to infect neonatal mice, which generally are more vulnerable to infection. Infection of 1-week-old mice with a pathogenic ZIKV isolate from Senegal resulted in death of approximately 30% of neonatal mice (Lazear et al., 2016). A subsequent study inoculated wild-type neonatal mice with a Puerto Rican clinical ZIKV isolate and demonstrated severe neurological disease, weight loss, and death in a subset of animals (Manangeeswaran et al., 2016). Infected neonatal wild-type mice exhibited infiltration of T cells into the central nervous system, similar to that observed in other models of neuroinvasive flavivirus infection.

Mouse models of ZIKV pathogenesis using clinical isolates in immunocompromised adult mice have shown that the virus can accumulate in the blood, spleen, brain, spinal cord, kidney, and eye. Studies of ZIKV pathogenesis using Ifnar1\(^{-/-}\) mice have recapitulated features of human disease, including placental infection and trans-placental transmission, neuroinvasive disease, and limb paralysis; the spinal cord disease in mice, however, likely does not represent autoimmune Guillain-Barre syndrome but rather is due to virus-mediated destruction of neurons. The testes of ZIKV-infected male Ifnar1\(^{-/-}\) mice also support high levels of ZIKV infection. In another mouse model of ZIKV pathogenesis, 6-week-old mice lacking three interferon regulatory transcription factors (IRF3, IRF5, and IRF7) were more vulnerable to infection with a Cambodian ZIKV clinical isolate and developed CNS infection that resulted in apoptosis of neural progenitor cells (Li et al., 2016b).

Studies in pregnant female mice inoculated subcutaneously with a French Polynesian clinical isolate have demonstrated that ZIKV infects different trophoblasts (including glycogen trophoblasts and spongiotrophoblasts and to a lesser extent mononuclear trophoblasts and syncytiotrophoblasts) and fetal endothelial cells of the placenta and then crosses the placenta to infect the fetal head (Miner et al., 2016a). Congenital infection of immunocompromised Ifnar1\(^{-/-}\) mice with ZIKV via a subcutaneous or intravaginal route

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resulted in similar abnormalities including intrauterine growth restriction and fetal demise (Miner et al., 2016a; Yockey et al., 2016). Pregnant wild-type C57BL/6 mice inoculated intravenously with a Brazilian clinical isolate did not have detectable virus in the fetus, most likely due to failure of the virus to replicate sufficiently in the placentas of these mice (Cugola et al., 2016). However, intravenous inoculation of SJL mice with unusually high doses (e.g., $\sim 10^{10} \text{--} 10^{11}$ PFU) of the same ZIKV strain induced intrauterine growth restriction and microcephaly in these animals (Cugola et al., 2016). In addition to differing routes of infection (subcutaneous versus intravenous), a distinguishing factor between the Ifnar1<sup>−/−</sup> and the SJL models was the gestational age at which the pregnant dams were infected with ZIKV. In the initial description of the Ifnar1<sup>−/−</sup> congenital infection model, the pregnant dams were infected subcutaneously at about embryo day 7 (E7), when the placenta and fetus may be vulnerable to damage. In comparison, Cugola et al. performed intravenous inoculation with ZIKV later in gestation (E10.5 to E12.5), which corresponds to a time of significant brain development in mice. A subsequent study found that subcutaneous inoculation of pregnant Ifnar1<sup>−/−</sup> mice with a Brazilian clinical ZIKV isolate at E9.5 caused intrauterine growth restriction and reduced fetus size without demise (Miner et al., 2016b), reminiscent of the phenotype observed in the SJL model. Consistent with these different infection phenotypes in mice, a range of ZIKV-induced congenital disease has been reported in humans, including intrauterine growth restriction, microcephaly, and miscarriage (Brasil et al., 2016a; Brasil et al., 2016b; van der Eijk et al., 2016). Although more studies are required to define the effects of gestational age on ZIKV pathogenesis during pregnancy, epidemiological data suggests that infection during the first and second trimesters in humans correlates with the most severe fetal disease (Pacheco et al., 2016).

Many of the findings from mice have been recapitulated in non-human primates, which will allow further testing of candidate therapies and vaccines in models that more closely resemble human disease (Abbink et al., 2016; Aliota et al., 2016b; Li et al., 2016c; Osuna et al., 2016). In rhesus macaques, ZIKV RNA was detected in plasma one day after subcutaneous inoculation with a French Polynesian ZIKV strain, and ZIKV RNA was isolated from the brain, cerebrospinal fluid, urine, and saliva for at least 3 weeks (Dudley et al., 2016). Another study in rhesus and cynomolgus macaques found that ZIKV RNA persisted in saliva and seminal fluids for at least 3 weeks after clearance of the virus from the peripheral blood (Osuna et al., 2016). Infection of pigtail macaques during pregnancy with a Cambodian clinical ZIKV isolate resulted in the development of fetal brain lesions with white matter hypoplasia (Adams Waldorf et al., 2016).

**ZIKV tissue and cell tropism**

One potential mechanism for observed microcephaly is that ZIKV preferentially infects and triggers apoptosis in neural progenitor cells (Dang et al., 2016; Onorati et al., 2016; Tang et al., 2016), although it also infects mature neurons to a lesser degree (Tang et al., 2016). This capacity of ZIKV to infect and injure progenitor cells may contribute to its neurodevelopmental impact on the brain. Indeed, direct intraventricular inoculation of the brains developing mouse fetuses from wild-type mice with ZIKV resulted in cortical infection and thinning, inhibition of neuroprogenitor cell differentiation, and microcephaly (Li et al., 2016a). Although this intraventricular inoculation route is not physiological, this
model confirmed the capacity for ZIKV to cause cell death and cerebral cortex disease in an animal model. As a corollary to observations from models of congenital infection, direct intracranial inoculation of ZIKV in postnatal wild-type mice caused depletion of proliferating cells in the stem cell compartment of the ventricular zone as well as disruption of corticospinal pyramidal neurons (Huang et al., 2016). Analogously, infection of human neurosphere organoid cultures in vitro with ZIKV impaired their growth and increased cell death (Garcez et al., 2016). Direct infection of neural progenitor cells may not be the only factor contributing to ZIKV-induced microcephaly. Infection of cranial neural crest cells may result in the production of inflammatory cytokines that act in a paracrine manner to deplete neural progenitor cell pools by promoting their apoptosis (Bayless et al., 2016; Dang et al., 2016).

In human studies, ZIKV RNA has been detected in both maternal and fetal tissues including cord blood, several placental cell types, amniotic fluid, and the developing fetal and neonatal human brain. ZIKV RNA also was detected in the brain and placenta of spontaneously aborted human fetuses in the first and second trimesters (Bhatnagar et al., 2017). After congenital infection of pigtail macaques with a Cambodian clinical ZIKV isolate, viral RNA was isolated from the maternal brain, eye, spleen and liver, with the highest amounts observed in the placenta (Adams Waldorf et al., 2016), suggesting that, as in mice and humans, ZIKV infects cells in the placenta of primates. In vitro infection studies of human placental cells have shown that ZIKV replicates in placental macrophages (Hofbauer cells), trophoblasts, and fetal endothelial cells and induces expression of antiviral genes (Quicke et al., 2016; Tabata et al., 2016). In one study, human trophoblasts isolated from a term placenta were relatively resistant to infection, in part due to inhibition mediated by type III IFN-λ (Bayer et al., 2016). Gestational age and genetic variation in host factors within the placenta (e.g., expression of virus attachment or immune restriction factors) may affect the relative vulnerability of placental cell types to ZIKV infection (Tabata et al., 2016).

Human and animal model studies have demonstrated that ZIKV infection can result in persistence of infectious virus and viral nucleic acid in several body fluids (e.g., semen, saliva, tears, and urine) and target organs including immune-privileged sites (e.g., eyes, brain, and testes) and the female genital tract. (a) Eyes. Studies in Ifnar1−/− mice found that ZIKV RNA is shed in tears (Miner et al., 2016b) and viral RNA can be detected in cornea, optic nerve, and neurosensory retina. Viral replication in eye-associated tissues in the mouse was corroborated with the discovery that ZIKV RNA and infectious virus can be recovered from human conjunctival fluid (Sun et al., 2016). (b) Female reproductive tract. One study found that ZIKV RNA is detectable in human cervical mucus at least 11 days after the onset of symptoms, after the virus had been cleared from blood and urine (Prisant et al., 2016). A subsequent case report described ZIKV RNA persistence in human vaginal secretions for more than 11 weeks (Murray et al., 2017). In vitro studies have shown that human uterine fibroblasts are susceptible to ZIKV infection (Chen et al., 2016), which suggests that uterine infection might contribute to impaired fetal development. (c) Male reproductive tract. Multiple studies have demonstrated infection of the male reproductive tract in mice (Chan et al., 2016; Govero et al., 2016; Ma et al., 2016). Infection of mice with a mouse-adapted African strain of ZIKV resulted in infection of spermatogonia and Sertoli cells, destruction
of the testes architecture, and reduction of motile sperm count (Govero et al., 2016). Several weeks after ZIKV infection, male mice had lower levels of the sex hormones testosterone and inhibin B compared to age-matched uninfected controls, and this was associated with reduced fertility (Govero et al., 2016). A subsequent study also demonstrated damage to the testis of ZIKV-infected mice, and suggested this could result in male infertility (Ma et al., 2016). Though chronic, persistence of ZIKV RNA in sperm and semen has been described in humans even up to six months (Barzon et al., 2016; Mansuy et al., 2016), such severe testicular injury has not yet been reported and thus, warrants further longitudinal evaluation in infected men.

Host restriction factors and candidate ZIKV receptors

Similar to other flaviviruses, it is anticipated that ZIKV tropism is restricted by multiple host factors including type I and type III IFNs as well as IFN-stimulated genes (ISGs) that are activated during infection. As examples of ISGs that restrict ZIKV infection, IFITM1 and IFITM3, members of a family of transmembrane antiviral proteins that limit pathogenesis of other flaviviruses (Brass et al., 2009), also restrict ZIKV replication in vitro and limit its ability to cause cell death (Savidis et al., 2016b). The identity of other ISGs contributing to the restriction of ZIKV infection pathogenesis remains to be determined.

At present, there is no established, physiologically relevant ZIKV receptor, although in vitro studies have suggested candidates that require validation in vivo. One candidate entry factor is AXL, a member of the TAM receptor family of cell surface receptor tyrosine kinases that interact with the phosphatidylserine-binding proteins Gas6 and Protein S, which in turn bind to the surface of enveloped viruses including flaviviruses (Bhattacharyya et al., 2013). AXL has been suggested to function as an attachment or entry factor for ZIKV based on in vitro studies in 293T cells, keratinocytes, and endothelial cells (Hamel et al., 2015; Retallack et al., 2016; Savidis et al., 2016a), and also because of a correlation with its expression on target neural progenitor cells (Nowakowski et al., 2016). Others have suggested that AXL expression may correlate with infection of the testis (Ma et al., 2016). However, a comparison of ZIKV infection in AXL-deficient and wild-type mice treated with an anti-Ifnar1 blocking antibody showed equivalent levels of virus in the serum, spleen, brain, testis, or eyes (Govero et al., 2016; Miner et al., 2016b); thus, AXL was not required as an entry factor for these tissues in mice. One caveat of these findings is that AXL engagement may enhance viral infections through its signaling functions by virtue of its ability to negatively regulate the IFNAR pathway (Bhattacharyya et al., 2013); as the studies in mice were performed in the setting of blockade of Ifnar1, this signaling activity might have been missed. Alternatively, it remains possible that AXL determines tropism for a limited number of cell types in a given tissue, which does not impact the overall viral burden. Lastly, another potential explanation may be species-specific differences in requirements of attachment/ entry factors by ZIKV such that other receptors have more dominant roles in mice. However, a recent study in human cells revealed that genetic ablation of AXL had no effect on ZIKV entry, replication, or cell death of iPSC-derived neural progenitor cells or cerebral organoids (Wells et al., 2016). Thus, although some studies suggest that AXL may function in cell culture as an important ZIKV entry factor, multiple in vivo and in vitro studies have demonstrated that AXL is not required for infection of many relevant tissues and cell types.
Another candidate receptor for ZIKV is TIM1, a glycoprotein that interacts with phosphatidylserine displayed on the viral membrane (Tabata et al., 2016). Indeed, TIM1 is expressed broadly on several ZIKV-infected cell types (e.g., Hofbauer macrophages, endothelial cells, and cytotrophoblasts) in the human placenta (Tabata et al., 2016). Whether TIM1 functions as a physiologically important attachment factor remains to be determined. Once confirmed as physiologically relevant, essential attachment or entry receptor(s) might serve as therapeutic targets to block ZIKV infection and pathogenesis.

**Humoral immunity to ZIKV**

ZIKV immune sera effectively neutralizes virus strains from both the Asian and African lineages (Dowd et al., 2016a), and prior infection with an Asian-lineage strain of ZIKV protects against heterologous infection with an African ZIKV strain (Aliota et al., 2016b), making it plausible that an effective vaccine can be developed against divergent ZIKV strains. Functional studies, epitope mapping, and cryo-EM structures of neutralizing anti-ZIKV antibodies bound to E protein have revealed multiple targets for vaccine design (Sapparapu et al., 2016; Wang et al., 2016; Zhao et al., 2016) (Figure 2A). Cross-reactive anti-DENV antibodies recognizing the envelope dimer epitope (EDE) (Dejnirattisai et al., 2015; Rouvinski et al., 2015) bind to and neutralize ZIKV infection (Barba-Spaeth et al., 2016) and protect AG129 mice from lethal ZIKV infection (Dai et al., 2016). Treatment with neutralizing human and mouse anti-ZIKV antibodies recognizing epitopes on the E protein dimer or in domain III can neutralize ZIKV infection and protect susceptible mice from lethal ZIKV challenge (Sapparapu et al., 2016; Zhao et al., 2016) (Figure 2A). Similarly, treatment of Ifnar1−/− mice with human monoclonal antibodies binding to distinct epitopes in E protein domains I, II, or III prevented weight loss and lethality during ZIKV infection (Wang et al., 2016). Neutralizing human antibodies also protected against congenital disease in a mouse model of in utero ZIKV infection (Sapparapu et al., 2016). Consistent with these findings, DNA plasmid adenovirus-vectorized, and inactivated virus vaccines protected rhesus macaques from ZIKV infection (Abbink et al., 2016; Dowd et al., 2016b) (Figure 2B).

During secondary DENV infection with a heterologous DENV serotype in humans, pre-existing cross-reactive anti-DENV antibodies from the first infection can promote the phenomenon of antibody-dependent enhancement (ADE) of infection (Dowd and Pierson, 2011), which is believed to promote greater infection in myeloid cells and more severe DENV disease (Halstead, 1979). Because of the high degree of structural and sequence similarity between ZIKV and DENV, antibodies produced to these flaviviruses can cross-react with one another (Dejnirattisai et al., 2016; Priyamvada et al., 2016; Stettler et al., 2016). These cross-reactive antibodies have implications for ZIKV vaccine design, as they could result in exacerbated disease with subsequent DENV infection. Indeed, cross-reactive anti-DENV antibodies can enhance ZIKV infection in cell culture (Charles and Christofferson, 2016; Dejnirattisai et al., 2016; Priyamvada et al., 2016) and reciprocally, cross-reactive human anti-ZIKV antibodies can enhance DENV infection in cell culture and in mice (Stettler et al., 2016). However, at present, it remains unknown whether ADE of ZIKV infection by anti-DENV antibodies occurs in humans or other experimental animal models. Although ADE is readily demonstrable in cell culture with many flaviviruses, more
detailed animal studies and epidemiological evidence are necessary to confirm whether clinically relevant antibody-dependent enhancement of ZIKV pathogenesis occurs in vivo.

**Conclusions**

Animal and human studies of ZIKV pathogenesis have revealed broad tissue and cell tropism for ZIKV as well as the capacity for the virus to cause severe end-organ disease in addition to placental and congenital infection. Established mouse and non-human primate models now serve as useful platforms to study ZIKV pathogenesis and to test candidate vaccines and therapies. Nevertheless, many unanswered questions remain with regard to optimal ZIKV antigens, the viral genetics of virulence, mechanisms of host restriction and immune evasion, the potential for ADE of ZIKV and DENV pathogenesis, as well as the long-term neurodevelopmental implications of congenital infection in humans. Given the exceptionally rapid pace of ZIKV research, we expect several of these questions to be answered soon.

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Figure 1. ZIKV tissue and cell tropism
Human studies and animal models (mice and non-human primates) have detected ZIKV in cells of the placenta including Hofbauer cells \((\text{in vitro} \text{ and in explanted human placental tissue})\), trophoblasts (mice, non-human primates, and humans), and endothelial cells \((\text{in vitro} \text{ in explanted human placental tissue and } \text{in vivo} \text{ in placenta of mice})\). Other ZIKV cellular targets include neuronal cell types including neural progenitor cells and mature neurons (mice, non-human primates, and humans), and astrocytes \((\text{in vitro} \text{ human cell cultures})\). In addition, ZIKV infects ocular tissues including the cornea, neurosensory retina, and optic nerve (mice), as well as the aqueous humor of the anterior chamber (humans). ZIKV also targets cells of the reproductive tract including spermatogonia, Sertoli cells, and Leydig cells (in the testis of mice), sperm (samples from mice and humans), and the vaginal epithelium (mice) and uterine fibroblasts \((\text{in vitro} \text{ infection of human samples})\). The extensive tropism results in ZIKV detection in multiple body fluids including conjunctival fluid or tears (mice and humans), saliva (non-human primates and humans), semen (mice, non-human primates, and humans), cervical mucus (humans), vaginal washings (mice and human) and urine (non-human primates and humans).
Figure 2. Targets for ZIKV vaccine design
A. Highly neutralizing mouse and human anti-ZIKV monoclonal antibodies bind to distinct epitopes in the E protein including the lateral ridge of domain III (e.g., ZV-67) (Zhao et al., 2016), a domain I–III interface epitope (e.g., Z23) (Wang et al., 2016), an EDE intra-dimer epitope (e.g., C10) (Zhang et al., 2016), domain I–II and domain II intra-dimer epitopes (e.g., Z3L1 and Z20, respectively) (Wang et al., 2016), and a domain II inter-dimer epitope (e.g., ZIKV-117) (Sapparapu et al., 2016). These epitopes represent candidate regions for ZIKV vaccine design. B. Vaccine approaches that are being developed for ZIKV include DNA plasmids encoding prM-E or E genes (Abbink et al., 2016; Dowd et al., 2016b), soluble E based proteins or peptides (Alam et al., 2016), or inactivated viral particles (Abbink et al., 2016). Vaccines should generate protective B and T cell responses for greatest efficacy. Potential concerns of ZIKV vaccines that will need to be resolved prior to deployment include possible induction of Guillain-Barré syndrome or sensitizing individuals to more severe future DENV infection due to the generation of cross-reactive antibodies that promote ADE.