Modeling Zika Virus-Associated Birth Defects in Nonhuman Primates

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In utero infection with Zika virus (ZIKV) during pregnancy can lead to the development of birth defects and postnatal deficits. A nonhuman primate (NHP) model of congenital ZIKV infection can help fill the gaps in knowledge where tissue studies are required to define viral pathogenesis and identify targets for therapeutic intervention. This model system has already identified critical features of ZIKV pathogenesis in congenital infection. Before translating these NHP studies to human clinical trials, we must understand the similarities and differences between human and NHP fetal immune system development, neural development, and infant assessment tools. Because of the overall similarity between fetal and infant development in humans and NHPs, this NHP model can complement human clinical trials by defining immune correlates of protection and evaluating therapeutic interventions.

Keywords. congenital Zika infection; nonhuman primate.

HUMAN CONGENITAL ZIKA VIRUS INFECTION, ASSOCIATED BIRTH DEFECTS, AND LATE-ONSET DEFICITS

In utero exposure to Zika virus (ZIKV) infection can result in a constellation of birth defects, including ocular anomalies, brain anomalies, cranial dysmorphologies, contractures, and neurologic sequelae including hearing loss [1–11]. Congenital ZIKV infection may have a significant impact on a generation of children, both in regards to the level of disability and the number of infants that are affected [9, 12–16]. The impact of congenital ZIKV infection reaches beyond the sum of birth defects identified at birth, because infants without obvious abnormalities at birth may develop impairments later in infancy. There is growing evidence that infants from ZIKV-infected pregnancies manifest decelerated head circumference growth months after birth [2, 17]. Although there are not yet longitudinal studies following the development of infants with congenital ZIKV infection given the recentness of the South American ZIKV outbreak, there may be development of deficits in the postnatal period after congenital ZIKV infection (delayed- or late-onset deficits) just as there are in other congenital infections, such as cytomegalovirus (CMV) [8]. Comprehensive epidemiological longitudinal studies of ZIKV-infected human infants are ongoing and will take years to define late-onset deficits that develop in the postnatal period [18–21].

DEFINING THE PATHOGENESIS OF BIRTH DEFECTS AND DELAYED-ONSET DEFICITS IN CONGENITAL VIRAL INFECTIONS

Animal models of pregnancy are necessary to perform studies that are both critical to improving the clinical outcomes of human infants and not possible in human studies. We must understand the similarities and differences of animals models to select models appropriate for studying the effect of in utero ZIKV infection on long-term neurodevelopmental outcomes and late-onset deficits. Congenital ZIKV infection research could benefit from decades of research on other congenital viral infections when determining how to best study these long-term developmental outcomes and late-onset deficits. Congenital CMV infection, similar to ZIKV, can result in the development of late-onset deficits, specifically sensorineural hearing loss (SNHL) [22, 23]. Congenital CMV infection is the most common cause of delayed-onset SNHL in children and can occur in infants who have asymptomatic infection at birth [24]. The pathogenesis of SNHL in congenital CMV infection is hypothesized to be secondary to direct cytopathic effects due to viral replication and localized inflammatory responses [25–27]. Antiviral therapy during infancy reduces the risk of hearing loss development, providing indirect evidence that ongoing CMV replication is important for the pathogenesis of hearing loss [28]. Determining whether postnatal microcephaly, which has been found in ZIKV-exposed infant [2, 17], or any other delayed-onset deficits are due to persistent ZIKV replication is critical to determining whether antiviral therapy in the postnatal period can decrease the risk of worsening outcomes after birth. Invasive tissue sampling is not possible in human clinical trials but would be possible in complementary nonhuman primate (NHP) studies to define the pathogenesis of these late-onset deficits and develop therapeutic interventions.
The biology of human pregnancy, including placental organization and gestational duration, is more closely paralleled by NHP pregnancy than by other animal models (reviewed in [29–33]). Nonhuman primates have a longer gestational duration than other animal models (mouse, guinea pig, sheep, pig) of human pregnancy (Table 1), which means a longer exposure time of the fetal compartment to ZIKV. In addition to a longer gestational time, humans and NHPs have a similar placental organization, which means that the passage of ZIKV from the maternal blood supply to the fetal compartment can be accurately modeled in an NHP model. Human and NHPs both have hemomonochorial type placentas. In a hemomonochorial type placenta, the maternal tissue layers disappear through erosion, leading to a direct connection between the chorion (the outermost fetal membrane) and maternal blood [34]. A hemomonochorial type placenta has only 1 layer of trophoblasts (specialized cells in the placenta) separating the maternal blood spaces from the fetal blood vessels [34, 35]. Guinea pigs also have a hemomonochorial placental type, but because their gestational duration is shorter than NHPs, they may not model aspects of gestational ZIKV infection as well. Other animal models of pregnancy lack a hemomonochorial type placenta (summarized in Table 1) [34, 35].

### The Future of Nonhuman Primate Models of Congenital Zika Virus Infection

The NHP model of congenital ZIKV infection has recapitulated many of the features of viral pathogenesis that are present during human congenital ZIKV infection, including prolonged maternal viremia, robust maternal neutralizing antibody responses, vertical transmission, fetal demise, uterine vasculitis and placental villous damage, periventricular brain lesions, and fetal retinal dysplasia [3, 10, 11, 36–47] (summarized in Table 2). The NHP model has advanced the understanding of ZIKV pathogenesis during pregnancy by suggesting a role of abnormal oxygen transport in placental dysfunction [39] and demonstrating how fetal neural progenitor cell population loss results in decreased brain volume in utero [38], discoveries that are impossible to reach from human clinical studies alone because of the need for invasive tissue sampling.

Thus far, the NHP studies have focused on the effects of gestational ZIKV infection on the fetus and placenta. There have not been any longitudinal studies that evaluate the effect of gestational ZIKV exposure during the postnatal period. Human clinical trials are now focusing on the long-term outcomes of infants after gestational exposure to ZIKV, and complementary NHP models should begin focusing on infant developmental outcomes and the drivers of pathogenesis in the postnatal period. Companion NHP clinical trials evaluating antiviral or immunotherapies in infants can help fill in the knowledge gaps, such as where persistent viral replication occurs. To translate findings from this NHP model into human clinical studies, we must understand the similarities and differences between human and NHP neural and immune system development. In addition, we must also understand the standardized tools available for NHP developmental assessments and understand how these compare with the standardized tools used in human infant assessments.

### Fetal and Infant Immune Responses to Congenital Zika Virus Infection

Because we have a limited understanding of the fetal and infant immune responses to congenital ZIKV infection, we should consider the immune responses to ZIKV in the nonpregnant state and immune responses to congenital CMV infection to help guide our investigations. From our earliest ZIKV studies...
in fetuses and infants, we have learned that ZIKV neutralizing antibodies are present at birth in both human and NHP fetuses after congenital ZIKV infection [37–39, 48, 49]. At birth, NHP infants have higher levels of inflammatory cytokines and chemokines in their peripheral blood and cerebrospinal fluid (CSF) compared with maternal peripheral blood and CSF [39]. Beyond these early studies, not much is known about the earliest immune responses to ZIKV in the fetus and in infancy.

If we look to congenital CMV infection to guide our congenital ZIKV infection immune response investigations, we should consider examination of CD4 and CD8 lymphocyte frequencies, gammadelta T-cell population frequencies, natural killer (NK) cell activating killer lectin-like receptor expression, immunoglobulin M antibodies, chemokine production, and cytokine production because these are all altered in CMV-infected fetuses compared to uninfected fetuses and may contribute to CMV pathogenesis in the fetal compartment [50–54].

If we look to immune responses to ZIKV infection in the nonpregnant state to guide our congenital ZIKV immune response investigations, we should examine the antibody responses in the fetal compartment and during infancy given the important role antibodies play in protection from infection and in virus control in preclinical vaccine and therapeutic trials. In ZIKV vaccine trials in nonpregnant NHPs, the development of neutralizing antibodies is critical to protection from challenge, and the antibody titer is closely associated with the likelihood of protection (reviewed in [29]). Passive administration of ZIKV-specific antibodies also prevents ZIKV infection in nonpregnant NHPs [55–57]. The role of antibodies in treatment of ZIKV infection during pregnancy is more complex because the administration of neutralizing antibodies to infected pregnant animals is not successful in limiting fetal infection [58]. Because the development of antibodies appears to be so important to the protection from infection, we should examine the role of ZIKV-specific antibodies in control of ZIKV infection during pregnancy. Nonhuman primates are a good model in which to study this aspect of the immune response because the cellular receptor responsible for transferring maternal antibodies to the fetus, the neonatal Fc receptor (FcRn), is expressed in the same cell types in the maternal-fetal interface, placental syncytiotrophoblasts, and fetal vessel endothelial cells [59]. Mice may not be as good of a model in which to study the role of antibodies in the fetal compartment given that the FcRn is expressed in the epithelial cells in the yolk sac endoderm [59], a different cellular location from humans and NHPs.
We must also understand the similarities and differences of the human and NHP immune system development during gestation and early infancy to translate findings from the NHP model to human clinical studies. One critical feature of both human and NHP immune system development is the exclusively intrauterine development of the immune system, with a complete complement of immune cells ready to function by the end of the second trimester [60, 61]. In contrast, neonatal mice and rodents have a 3-week period after birth until the immune system is ready for immunoreaction [60]. Buse [60] summarized key phases of immune system development in NHPs and showed that in humans and NHPs, hematopoietic stem cells are formed when gestation is 12%–13% underway; in contrast, these cells form in mice who are 36%–48% underway with gestation [60]. Cells migrate to the fetal liver and thymus between completion of 13%–21% gestation in primates and completion of 47%–76% in mice [60]. The thymus is developed when gestation is 15%–21% completed (compared with mice at 52% of gestation completed), and the bone marrow is established in primates at 30%–34% of gestation completed (compared with 76% completed in mice) [60]. This early development of the immune system in humans and NHPs compared with mice prepares the primate immune system for immunoreaction in the prenatal period, whereas the mouse immune system is only ready for immunoreaction in the postnatal period [60–62]. In addition, although primate functional memory cell pools are established in the postnatal stage before weaning, mice only establish these functional pools of memory cells after weaning [60]. The similarities in the immune system development between humans and NHPs suggest that the earliest immune responses to in utero ZIKV infection can be accurately modeled in NHPs. One difference noted between humans and NHP fetal tissues was that although B cells, NK cells and CD4+ T cells were detected in these tissues, CD8+ T cells were not observed before birth [60]. Overall, however, the immune system similarities between humans and NHPs are likely very important when modeling gestational exposure to prolonged maternal viremia during many weeks of gestation, as happens in pregnant humans and NHPs [36, 40, 41, 63, 64].

**FETAL AND INFANT NEURODEVELOPMENT IN CONGENITAL ZIKA VIRUS INFECTION**

Many ZIKV-associated birth defects are seen in the brain [2, 17, 65], highlighting the importance modeling of ZIKV pathogenesis in a central nervous system that develops in a similar way to humans to better understand ZIKV-associated birth defects and postnatal deficits. Brain neurodevelopmental abnormalities arise when ZIKV targets neural progenitor cells and glial cells in the fetal brain, which causes cell death and reduced neural progenitor cell proliferation [66]. This conclusion was reached using evidence collated from multiple model systems, including neural stem cell cultures, 3D neurospheres and human brain organoids, human fetal tissue cultures, and animal models [66]. Similar to the findings of human ZIKV-associated brain abnormalities, NHP models of congenital ZIKV infection have identified central nervous system abnormalities including fetal brain lesions [37, 38, 40] and histopathology in sections of the eye that are part of the central nervous system, the retina [41], and optic nerve [36], as summarized in Table 2. Both Adams Waldorf et al [38] and Martinot et al [40] described abnormalities in the neural progenitor cell population in fetal brains. Knowledge gaps remain regarding viral neuropathogenesis during gestation and in early infancy. These include whether late-onset microcephaly is secondary to viral replication and/or neural progenitor cell death and whether antiviral therapy can prevent the development of postnatal deficits. These questions are difficult to study in humans given the impossibility of sampling brain tissues in liveborn infants and impossible to study in in vitro models. Nonhuman primate models of congenital ZIKV infection can help bridge these knowledge gaps, but first we must consider whether the neurodevelopment of human and NHP brains, specifically the development of neural progenitor cell populations, are similar.

Neural progenitor cell population development has been well studied in human fetuses [67], and NHP brain development follows a similar trajectory [68]. Neural progenitor cells first develop between 13 and 20 days after conception in human embryos in the epiblast [67]. The neural tube forms 20–27 days after conception in humans [67] and 18–24 days after conception in NHPs [69]. Neural progenitor cells form a single layer of cells that line the center of the neural tube [67]. As the brain becomes larger and more complex, the shape of the hollow tube also changes, eventually forming the ventricular system of the brain [67]. Because the neural progenitor cells are located in the region that will become the ventricles, this neural progenitor cell-rich region is called the ventricular zone [67]. The next steps in fetal brain development occur as the smooth lissencephalic structure gradually develops the characteristic mature pattern of gyral and sulcal folding [67]. As neurons are produced in the ventricular zone, they migrate away to form the neocortex in an orderly fashion, forming a 6-layered cortical mantle [67]. After birth, neurogenesis decreases and only occurs in the subventricular zone and in the subgranular layer of the dentate gyrus of the hippocampus in humans [67]. In humans, neurogenesis from the subventricular zone is largely completed by 2 years of age [70] and continues throughout adulthood in the subgranular layer of the dentate gyrus of the hippocampus [71]. In NHPs, neurogenesis in the dentate gyrus continues until adulthood; the duration of neurogenesis in the subventricular zone is less well defined [71]. These similarities in development of the neuronal progenitor cell populations is key when defining the effect of ZIKV pathogenesis on central nervous system development. One key difference in postnatal neurogenesis between humans and NHPs is the location where neural progenitor cells from
the subventricular zone migrate. In NHPs, the neural progenitor cells from the subventricular zone migrate to the olfactory bulb and in humans they migrate to the nearly striatum [72]. The significance of this difference is not known. Nonhuman primate studies of ZIKV-associated birth defects in the postnatal period should take into account this difference when translating findings to human infants. In summary, the embryological events leading to formation of the neural progenitor cell population and their persistence in the postnatal period is similar in humans and NHPs, making the NHP model of ZIKV-associated central nervous system birth defects and late-onset deficits a good model of human neurodevelopment.

ASSESSMENTS OF INFANT DEVELOPMENT IN HUMANS AND NONHUMAN PRIMATES

Human infants can have developmental delays, hearing loss, and decreased visual function after congenital ZIKV infection [9, 73]. The only study to evaluate NHP infant development after ZIKV infection to date found that NHP infants infected with ZIKV in the postnatal period had abnormal anxiety development [74]. Existing NHP models of congenital ZIKV infection should begin assessing these clinical outcomes in interventional studies to translate the meaningfulness of ZIKV pathogenesis into predictive outcomes. Tools are already in place to assess NHP infant neurodevelopment, vision, and hearing because NHPs have been used to study infant and child development for decades [75]. Human infant neurobehavioral assessments include the Brazelton Neonatal Behavioral Assessment Scale, the Bayley Scales of Infant Development, and others [76]. The human Brazelton Neonatal Behavioral Assessment Scale was adapted for use in NHP infants by Schneider and Suomi [77]. There are other neurodevelopmental assessments that have been specifically developed for NHP infants [78], including the Human Intruder Test, which measures anxiety in infant NHPs [79] and was used by Mavignier et al [74] to measure anxiety development after postnatal ZIKV infection in NHPs. Hearing studies also have a rich history in NHP research [80, 81]. Human infant hearing is assessed using otoacoustic emission testing and auditory brainstem response testing [82]. Auditory brain response testing has also been used as a hearing assessment tool in infant NHPs [83]. Visual function and development assessment tools have also been used frequently in NHP studies. Human infant visual function is assessed with visual acuity and electrophysiological studies. Visual acuity studies, such as preferential looking tests, assess where infants prefer to look, based on the psychological premise that infants prefer to focus on patterned rather than homogeneous fields [84]. Electrophysiological studies, such as electroretinography and visual evoked potentials, can define dysfunction when there is a normal fundus examination but apparently limited visual acuity, by measuring electrical activity in the retina and visual cortex [85, 86]. Both visual acuity and electrophysiological studies have been have both been adapted for use in infant NHPs [87–89]. These neurodevelopmental, hearing, and visual function assessments that have been established in other applications of NHP research should be adapted for use in the congenital ZIKV infection field to evaluate the effect of therapeutic interventions.

CONCLUSIONS

The use of NHPs to model fetal and infant development in congenital ZIKV infection is an emerging effort. Early studies of congenital ZIKV infection in this NHP model have expanded the understanding of congenital ZIKV infection beyond what has been learned in human clinical studies. For instance, associating neural progenitor cell population abnormalities in the fetal brain with corresponding magnetic resonance images has not been done in human clinical trials and likely will not be able to be done given the limitation of sampling human infant brain tissue. Infant immune response studies and neurodevelopmental assessments in this NHP model of congenital ZIKV infection are now being initiated. Nonhuman primate models will complement human clinical trials and will be especially useful in defining immune correlates of protection and evaluating therapeutic interventions that limit the development of birth defects during gestation and developmental deficits in the postnatal period.

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