A previously undetected pathology of Zika virus infection

Kimberly M Christian, Hongjun Song & Guo-li Ming

In a nonhuman primate model of Zika virus infection, structural and cellular pathology deficits that could have a long-lasting impact on neural development and neurocognitive function are detected in offspring of infected mothers.

It has been two years since the World Health Organization (WHO) declared concern over the rising number of cases of microcephaly in regions with an active Zika virus (ZIKV) outbreak. Since then, considerable progress has been made in understanding the neurological consequences of ZIKV infection[1]. In 2016, we and others[1–3] showed that ZIKV is neurotropic and can directly and productively infect human neural progenitor cells (NPCs) in both 2D and 3D cell culture models of cortical development, resulting in reduced proliferation and increased death of neural progenitors. In parallel, early models of ZIKV infection in pregnant mice showed direct infection of fetal NPCs, viral replication in the embryonic brain, and microcephalic-like outcomes in offspring at birth[4]. Nonhuman primate models of ZIKV infection were also developed to better recapitulate lengthy gestational periods and physiological features of human embryonic development and immunological responses[5]. Together with clinical and epidemiological reports, these studies provided strong evidence that ZIKV causes microcephaly and has the potential to contribute to numerous neurodevelopmental deficits[6]. Congenital Zika syndrome has now been defined as a spectrum of abnormalities that range far beyond microcephaly to include other severe impairments, such as craniofacial disproportion, ocular anomalies, and intracranial calcifications, but the nature and extent of potential Zika-related pathology is still not fully understood. Here, Adams Waldorf *et al.*[7] further study offspring of ZIKV-infected pigtail macaques and identify indications of neurological damage in the absence of microcephaly.

In this study, Adams Waldorf *et al.*[7] inoculated five pregnant pigtail macaques with either the Cambodian or Brazilian strain of ZIKV to track the emergence of pathology in neurogenic regions *in utero* and to identify any notable effects on the brain at full-term delivery. Monkeys were infected at different time points during pregnancy, ranging from day 60 to day 119 of the typical 170-d gestational period (**Fig. 1a**). Three of the monkeys also received a monoclonal dengue virus antibody to model the effects of pre-exposure to another flavivirus. However, as Adams Waldorf *et al.*[7] acknowledge, the small number of monkeys and multiple treatment conditions in this experimental design preclude any definitive conclusion about whether dengue antibodies potentiate the deleterious effects of ZIKV infections. Following inoculation with ZIKV, none of the fetuses developed microcephaly, and weekly ultrasounds did not reveal any gross fetal abnormalities until gestational day 100. After this point, there was a decrease in the ratio of noncortical tissue to overall brain volume, rather than the changes in absolute cortical volume that are usually implicated in microcephaly, in fetuses of inoculated pregnant macaques as compared to fetuses of mock-infected pregnant macaques. In accordance with the findings from one fetus in their previous study[6], neuropathology, including gliosis and changes in ventricular structures, was observed to varying degrees in an additional three fetal brains (**Fig. 1b**). Interestingly, these changes were not observed in a fifth fetal brain from a monkey that had one of the highest viral loads but negligible immunological activity, as determined through the number of natural killer cells in the placenta and maternal blood, illustrating the range of pathological and immunological responses to ZIKV infection.

Given the preferential targeting of human NPCs by ZIKV that was reported in previous studies[2], the authors focused on two regions that house NPCs and support postnatal neurogenesis: the subventricular zone (SVZ) of the temporal cortex and subgranular zone (SGZ) of the dentate gyrus in the hippocampus. Interestingly, there was decreased proliferation of NPCs and a trend toward fewer NPCs overall in the SVZ, and there were fewer actively proliferating NPCs and a disorganized topography of NPCs and dysmorphic immature neurons in the SGZ (**Fig. 1b**).

Unlike the changes in volumetric ratios that could be readily detected *in utero*, the observed brain-region-specific anatomical and cellular abnormalities revealed upon histological examination, such as discontinuities in the granule cell layer, disordered strata, and misalignment of cellular processes, would not be clinically detectable in human infants. However, these aberrant developmental changes could have widespread and long-lasting effects on brain function. For example, the hippocampus is a critical substrate for learning and memory and a site of constitutive neurogenesis in many adult mammals, including humans[8]. Functional and structural disturbances of this region have been implicated in impaired cognition, psychiatric disorders, and epilepsy[9]. The persistent vulnerability of both the SVZ and SGZ neurogenic regions to ZIKV was suggested in an earlier study in mice that showed targeted infection of adult NPCs, leading to increased cell death and reduced proliferation in the mature brain[10].

The fact that ZIKV infections can often go unnoticed or trigger only mild and transient flu-like symptoms in otherwise healthy adults has collateral effects on public health. First, it increases the risk that pregnant women or young children are unknowingly infected or exposed to infected individuals. Second, the individual variability in resistance to ZIKV in adults makes it tempting to assume that fetuses or infants without any overt morphological or structural abnormalities are likewise protected from any long-term consequences of ZIKV exposure. However, this study indicates that undetected changes could be happening and reveals the dangers of the assumption that they are not, particularly given the limits of our current technology to detect subtle morphological disturbances *in vivo*.

It is well known that human brain development extends for many years beyond birth,

Kimberly M. Christian, Hongjun Song and Guo-li Ming are at the Department of Neuroscience and Mahoney Institute for Neurosciences, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. Hongjun Song and Guo-li Ming are also at the Department of Cell and Developmental Biology and the Institute for Regenerative Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. Additionally, Hongjun Song is at Epigenetics Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.
e-mail: gming@pennmedicine.upenn.edu

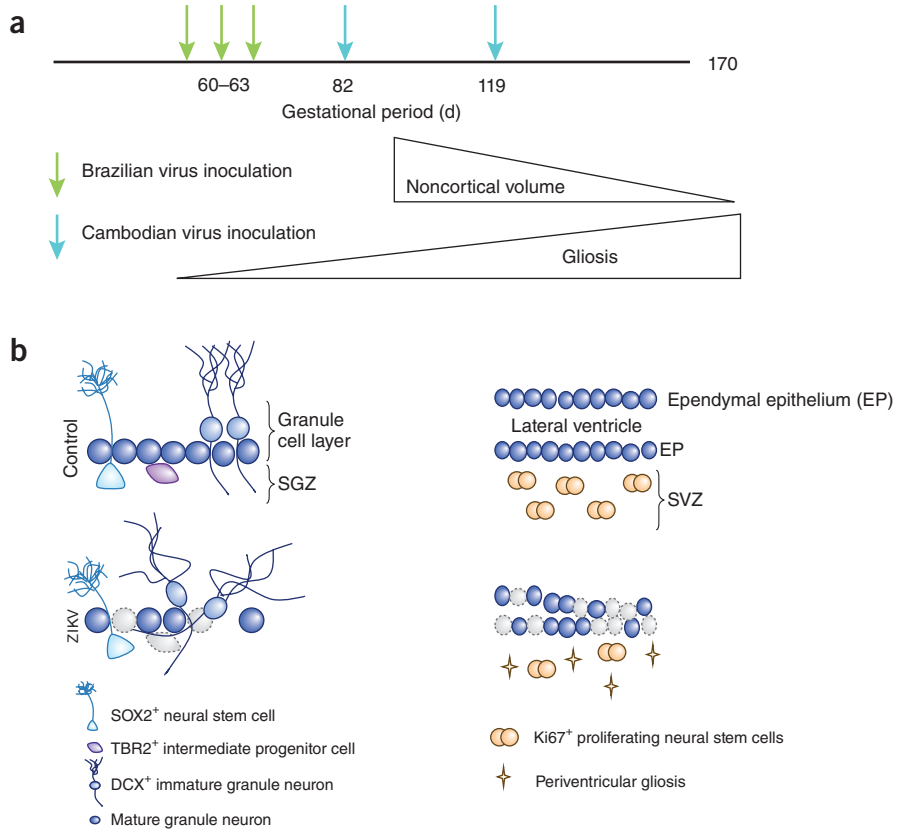

Figure 1 *In utero* exposure to ZIKV perturbs fetal brain development. (**a**) Adams Waldorf *et al.*[7] inoculated pregnant pigtail macaques with either a Cambodian or Brazilian strain of ZIKV at different time points in the gestational period, which led to gliosis that was more pronounced following later-stage infections and gradual decline in the relative volume of noncortical regions after 100 d *in utero*. (**b**) Adams Waldorf *et al.*[7] identify abnormal histopathology observed in neurogenic regions, including the subgranular zone (SGZ) (left), which shows disorganized neural stem cells and immature granule neurons and a loss of neuronal progenitors, and the subventricular zone (SVZ) (right), which shows a loss of ependymal cells, fusion of ventricular surfaces, decreased cell proliferation, and increased gliosis.

increasing the window of susceptibility to agents and conditions that can derail the organization of neural circuits and compromise brain function. Based on early surveillance guidelines to report cases of confirmed microcephaly, the Zika Outcomes and Development in Infants and Children (ZODIAC) study was organized for longitudinal monitoring of microcephalic infants. It remains an important effort and has revealed a number of debilitating symptoms in children up to 24 months of age[11]. Other recent studies have indicated that the number of infants and children at risk for Zika-related pathology could be much greater than previously appreciated. In a recent study of 37 children diagnosed with congenital Zika syndrome, brain calcifications were observed in computed tomography scans shortly after birth but had largely diminished or disappeared in follow-up scans taken approximately 1 year later[12]. Strikingly, the resolution of this structural abnormality was not correlated with improved neurological outcomes. Together, these results underscore the importance of assessing cognitive development and neural function in all infants and young children exposed to ZIKV because of the potential for sustained pathology in neurogenic regions that falls below the threshold of detection using standard clinical measures. The limited availability of human tissue to perform histological analyses at different developmental stages also reinforces the need to use animal and stem cell models, as illustrated in this study, to recapitulate the acute and chronic effects of ZIKV infections on key milestones in later stages of brain development, such as gliogenesis, myelination, and synapse formation, and ultimately in circuit formation and behavior. By quantifying and highlighting subtle anomalies in constitutively neurogenic regions in the developing brain, this study raises new concerns about the long-term effects of exposure to ZIKV *in utero* and early childhood.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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