The impact of Zika virus on the developing brain

Something new under the sun

William B Dobyns, MD
Departments of Pediatrics and Neurology
University of Washington, and
Center for Integrative Brain Research
Seattle Children's Research Institute
Seattle, WA
Brazil

**LETTERS**

Zika Virus Outbreak, Bahia, Brazil

Gustavo S. Campos, Antonio C. Bandeira, Silvira I. Sardi

Authors affiliations: Federal University of Bahia, Salvador, Bahia, Brazil (G.S. Campos, S.I. Sardi); Hospital Alineka, Salvador (A.C. Bandeira)

**DOI**: dx.doi.org/10.1128/mBio.015847

**To the Editor**: Zika virus (ZIKV) is a mosquito-borne flavivirus related to yellow fever virus, dengue virus (DENV), and West Nile virus (WNV). It is a single-stranded positive RNA virus (10,794 nt genome) that is closely related to the Flaviviridae family and is transmitted primarily by Aedes spp. mosquitoes, including *Ae. aegypti*, *Ae. albopictus*, *Ae. hasei*, and *Ae. scutellaris*. The virus was identified in rhesus monkeys during a study of the Zika virus in Uganda in 1947 and was reported in humans in 1952 (1).

In 2007, an outbreak of ZIKV was reported in Yap Island, Federated States of Micronesia (2). ZIKV also caused a major epidemic in the French Polynesia in 2013–2014 (3), and New Caledonia reported imported cases from French Polynesia in 2015 and reported an outbreak of the disease in 2016 (4).

A new challenge has arisen in Brazil with the emergence of ZIKV and co-circulation with other arboviruses (i.e., DENV and chikungunya virus [CHIKV]). We report ZIKV infection in Brazil associated with a recent ongoing outbreak in Camaçari, Bahia, Brazil, an area of high case incidence characterized by macaronesia racch, fever, myalgia/articular/cranioconjunctivitis.

On March 26, 2015, serum samples were obtained from 234 patients (Table 1) at the Santa Helena Hospital in Camaçari who were given a presumptive diagnosis of an acute viral illness by emergency department physicians. These patients were given treatment for a dengue-like illness, and blood samples were obtained for complete blood counts and serologic testing by using an ELISA specific for IgG and IgM against DENV. Serum samples were analyzed at the Federal University of Bahia by reverse transcription PCR (RT-PCR) to detect DENV, CHIKV, WNV, Mayaro virus, and ZIKV. In brief, serum samples were subjected to RNA extraction by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). RNA was reverse transcribed by using the SuperScript III Reverse Transcript Kit (Invitrogen, Carlsbad, CA, USA) and subjected to PCR specific for DENV (5) CHIKV (6), WNV (7) and Mayaro virus (8). A positive RT-PCR for a partial region of the envelope gene with primers ZIKVENF and ZIKVENVR (positions 379–450) under the media. Zika virus infection was confirmed in 18% of the population tested (9).

**Possible Association Between Zika Virus Infection and Microcephaly — Brazil, 2015**

Larissa Schuler-Freitas, PhD, (10); Ednéa M. Barreto, PhD, (10); Jair N.L. Ferreira, MD, PhD, (11); Daniel E.G. Lourenço, PhD, (10); Denise P. Cavalcanti, PhD, (10); André Prosperi, Maria Júlia R. Dutra, M.D., (10); Jean José Leal Neto, MD, PhD, (10); Heidy Mendes de Piza Neto, PhD, (10); Heidy Mendes de Piza, PhD, (10); Eliza de Medeiros, PhD, (10); Maria Teresa V. Santos, PhD, (11); Brazilian Medical Genetics-Society Zika Embraarity Task Force(11)

**On January 22, 2016, this report was posted as an MMWR Early Release on the MMWR website (http://www.cdc.gov/mmwr).**

In early 2015, an outbreak of ZIKV, a flavivirus transmitted by Aedes mosquitoes, was identified in northeast Brazil, an area where dengue virus was already circulating. By September, reports of an increase in the number of infants born with microcephaly in Zika virus–affected areas began to emerge, and Zika virus RNA was identified in the amniotic fluid of two women whose fetuses had been found to have microcephaly by prenatal ultrasound. The Brazil Ministry of Health (MoH) established a task force to investigate the possible association of microcephaly with Zika virus infection during pregnancy and a registry for incident microcephaly cases (head circumference ≤2 standard deviations [SD] below the mean for sex and gestational age at birth) and pregnancy outcomes among women suspected to have had Zika virus infection during pregnancy. Among a cohort of 35 infants with microcephaly born during August–October 2015 in eight of Brazil’s 26 states and reported to the registry, the mothers of all 35 had lived in virus–affected areas during pregnancy. 23 (66%) infants had severe microcephaly (broad circumference ≤3 SD below the mean for sex and gestational age), 17 (49%) had at least one neurologic abnormality, and among 27 infants who had neuroimaging studies, all had abnormalities. Tests for other congenital infections were negative. All infants had a head circumference as part of the evaluation and confirmed microcephaly (head circumference ≤3 SD below the mean for sex and gestational age). The MoH confirmed an increase to birth prevalence of microcephaly in northeast Brazil, compared with previously reported estimates (approximately 6.5/10,000 live births), which are based on review of birth certificates and include descriptions of major congenital anomalies. The MoH rapidly established a microcephaly registry in Brazil. On November 17, 2015, the MoH reported the increase in microcephaly cases, and possible association of microcephaly with Zika virus infection during pregnancy on its website(11) and the Pan American Health Organization (PAHO) published an alert regarding the increase in occurrence of microcephaly in Brazil (12).

In December, PAHO published the identification of Zika virus RNA by reverse transcription–polymerase chain reaction (RT-PCR) in amniotic fluid samples from two pregnant women whose fetuses were found to have microcephaly by prenatal ultrasound, and the identification of Zika virus RNA from multiple body tissues, including the brain, of an infant with microcephaly who died in the immediate neonatal period (13). These events prompted new alerts from the MoH, the European Centre for Disease Prevention and Control (14), and CDC (15) concerning the possible association of microcephaly with the recent outbreak of Zika virus infection.

A comprehensive protocol for notification and investigation of all infants with microcephaly and all women with suspected Zika virus infection during pregnancy was developed by the MoH and implemented nationwide. In addition, the Brazilian Society of Medical Genetics established the Zika Embraarity Task Force (SBGM-ZEFTF), which includes clinical geneticists, obstetricians, pediatricians, neurologists, and radiologists, to review all incident cases of microcephaly as well as all infants born to mothers with suspected Zika virus infection during pregnancy. Task force members collect data concerning the pregnancy (including exposure history, symptoms, and laboratory testing), physical examination of the infant, and any additional studies using a standardized spreadsheet. Microcephaly was defined as neonatal head circumference ≤2 SD below the mean for gestational age and sex of the infant at birth. Infant with Zika virus infection is difficult to confirm retrospectively because...
Zika in Brazil

Emergence of Congenital Zika Syndrome: Viewpoint From the Front Lines

Zika, a mosquito-borne flavivirus discovered in Uganda in 1947, remained obscure until its emergence in Micronesia in 2007. Six years later, it arrived in French Polynesia and other islands in the South Pacific (1). The virus was first detected in Brazil in early 2015 and has now spread throughout South and Central America and the Caribbean (2). Infection often remains unrecognized because it is either asymptomatic (73% to 80%) or has a nonspecific presentation of rash and fever. The first suggestion that Zika virus causes more than a self-limited illness was during the French Polynesian outbreak, when incidence of Guillain-Barré syndrome increased 20-fold (3). Likewise, a cluster of cases of this syndrome was identified in Brazil after the introduction of Zika virus (4).

From July to September 2015, several months after the introduction of Zika virus into northeastern Brazil, obstetricians noticed an increased number of fetuses with congenital malformations during ultrasound screening. By October, the number of newborns with microcephaly had increased significantly in this area, according to birth registry data from previous years. Microcephaly had now risen in other regions along with the spread of Zika virus. To date, more than 4,000 cases have been reported (Figures 1 and 2).

That Zika virus is the cause of the large number of microcephaly cases identified during the epidemic remains presumptive (5). Brazilian researchers first noted the virus’s potential association with microcephaly when they investigated a newborn with this condition, who died soon after birth and was found to have detectable virus in tissues. Subsequently, Zika virus RNA was detected in additional cases of fetuses and stillbirths with congenital malformations (6-8). To date, the strongest evidence of the correlation between Zika virus and microcephaly is a circumstantial link between the spatial and temporal patterns of these infections and the appearance of microcephaly. In addition, this condition was retrospectively identified in infants born during the 2013 outbreak in French Polynesia. Despite these observations, investigators have not determined a definitive association between Zika virus and microcephaly cases in the Brazilian outbreak, most of which have been live-born infants.

Our investigation is still in progress; however, we have gained insight into the scope and severity of microcephaly due to presumed congenital Zika syndrome.
Effects of Zika virus in fetal brain

- ZIKV is trophic for neural stem cells
  - Embryonic stem cells: ~15-20%
  - Neural stem cells: ~70-85%
  - Neurons: ~10-20%

- Neural stem cells and radial glia are the same cells

- Zika virus causes far more extensive brain injury than just “microcephaly”
ZIKV in human NPC

**Cell Stem Cell**

**Brief Report**

**Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth**

Hengli Tang,1,2,3,4 Christy Marnock,1,2,3 Sarah C. Ogden,1,2,3 Zhengwei Wen,2,3,4 Yuchen Qian,2,3,4 Yuling Li,2,3,4 Bing Yao,1,2,3,4 Jae-Hyun Shin,2,3,4 Feiyan Zhang,2,3,4 Emily N. Lee,2,3,4 Kimberly M. Christian,2,3,4 Ruth A. Dilley,2,3,4 Peng Jin,2,3,4 Hongjun Song,2,3,4 and Guo-gui Ming5,6,7,8

1Department of Biomedical Science, Florida State University, Tallahassee, FL, 32306, USA.
2Institute for Cell Engineering.
3Department of Radiology.
4Biomedical Engineering Graduate Program.
5Cellular and Molecular Medicine Graduate Program.
6Psychiatry Department of Neuroscience.
7Kroc Neuroscience Discovery Institute.
8Department of Psychiatry and Behavioral Sciences.
9Kroc Institute for Brain Health.

**Summary**

The suspected link between infection by Zika virus (ZIKV), a re-emerging Flavivirus, and microcephaly is an urgent global health concern. The direct target cells of ZIKV in the developing human fetus are not clear. Here we show that a strain of the ZIKV, MR766, serially passaged in monkey and mosquito cells efficiently infects human neural progenitor cells (hNPCs) derived from induced pluripotent stem cells. Infected hNPCs further release infectious ZIKV particles. Importantly, ZIKV infection increases cell death and dysregulates cell-cycle progression, resulting in attenuated hNPC growth. Global gene expression analysis of infected hNPCs reveals transcriptional dysregulation, notably of Wnt and Notch pathways. Our results identify Wnt signaling as a target in human neural progenitors. In addition, we identify a monocytes/macrophages as a potential mechanism of ZIKV and provide a platform for future studies.

**Figure 1.** ZIKV Infects hPSC-Derived Neural Progenitor Cells with High Efficiency
Radial glia are neural stem cells

Department of Neurology, University of California, San Francisco, CA 94143, USA

Radial glia are neural stem cells

Deborah C. Nathan, T. M. Koh, and D. W. Knoblich

Methods

Neural stem cells are derived from the radial glial cell lineage and can be cultured in a self-renewing state in vitro. These cells exhibit a characteristic bipolar morphology with a long, slender process extending from the bottom of the dish to the surface. The cells are positive for markers such as nestin and glial fibrillary acidic protein (GFAP). To test the self-renewing capacity of these cells, they are grown in a medium containing basic fibroblast growth factor (bFGF) and heparin. Under these conditions, the cells form a self-renewing population that can be serially passaged for extended periods of time. This population is maintained in a state of self-renewal and can give rise to progenitor cells that can differentiate into multiple cell types, including neurons and astrocytes.

Results

The self-renewing population of neural stem cells exhibits a characteristic bipolar morphology and expresses markers of radial glia. The cells are positive for nestin, GFAP, and other radial glia markers. Furthermore, they can be serially passaged for extended periods of time in the presence of bFGF and heparin. This self-renewing population can give rise to progenitor cells that can differentiate into multiple cell types, including neurons and astrocytes.

Conclusions

These data support the hypothesis that radial glia are neural stem cells and provide evidence for the existence of a self-renewing population of neural stem cells that can give rise to progenitor cells with the potential to differentiate into multiple cell types. This work has important implications for the study of neural development and regeneration.
Congenital Zika syndrome with FBD
Fetal brain disruption sequence

- Profound MIC
- Overlapping sutures frontal-parietal bones
- Overriding occipital bone
- Scalp rugae

The fetal brain disruption sequence is a recognizable pattern of defects that includes moderate to profound microcephaly, overlapping sutures, occipital bone prominence, and scalp rugae. The condition is postulated to arise from partial brain disruption during the second or third trimester with subsequent fetal skull collapse resulting from decreased intracranial hydrostatic pressure. Proposed causes include prenatal viral or parasitic infections and vascular disruptions. We report seven infants with the fetal brain disruption sequence. Two of these patients died. A changing phenotype with time was seen in three. Recognition of this phenotype is critical because the condition has a uniformly poor prognosis for infants but the recurrence risk in future pregnancies is low. (J Perinat 1995;15:363-4.)

Russell et al. previously reported three infants with a recognizable pattern of defects that included severe microcephaly, overlapping sutures, occipital bone prominence, and scalp rugae in addition to the severe microcephaly (Figs. 1 and 2). Persistent and disappeared by 6 months. Radiographs showed a reduced midline overriding sutures and a broad biparietal occipital bone (Fig. 3). The CT scan showed ventriculomegaly and various degrees of calvaria defect (Fig. 4); one infant had classic hydrops occurred in all seven pregnancies (1). Maternal untreated goiter (1), maternal alcohol or drug (4), maternal cigarette smoking (3), and immunologic maternal cytokines were found in two patients. Two infants were diagnosed with chronic fetal anemia (1), intrauterine growth retardation (2), and decreased fetal movement (1). Evidence consistent with septicemia included histopathologic examination of placental and fetal tissues from all seven patients (unlabeled). No maternal evidence of intrauterine infection was noted. Three patients were alive at more than 5 months of age, with no follow-up, and two showed serious retardation. Two of the affected infants died at 3, 4, and 4 years of age, complete loss of overlapping sutures and scalp rugae in addition to microcephaly.
Congenital Zika syndrome - CT
Zika-24
Congenital Zika syndrome - MRI
## Congenital Zika syndrome DATA

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>Group 1 MIC</th>
<th>Group 2 Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal viral illness</td>
<td>38/56 (68%)</td>
<td>7/9 (78%)</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; trimester</td>
<td>26/56 (46%)</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; trimester</td>
<td>09/56 (15%)</td>
<td>4/9 (44%)</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; trimester</td>
<td>02/56 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>Serologic testing for ZIKV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serologic or genetic testing</td>
<td>10/57 (18%)</td>
<td>7/9 (78%)</td>
</tr>
<tr>
<td>Positive results for ZIKV</td>
<td>10/10 (100%)</td>
<td>2/9 (22%)</td>
</tr>
<tr>
<td>Gestational ages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Term births</td>
<td>50/57 (88%)</td>
<td></td>
</tr>
<tr>
<td>Preterm births</td>
<td>07/57 (12%)</td>
<td></td>
</tr>
</tbody>
</table>
## Congenital Zika syndrome DATA

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>Group 1 MIC</th>
<th>Group 2 NL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth and OFC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC –2 to –3 SD</td>
<td>11/57 (19%)</td>
<td>0/9</td>
</tr>
<tr>
<td>MIC below –3 SD</td>
<td>46/57 (81%)</td>
<td>0/9</td>
</tr>
<tr>
<td>IUGR</td>
<td>3/48 (6%)</td>
<td>0/9</td>
</tr>
<tr>
<td>Neurologic features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spasticity</td>
<td>47/50 (94%)</td>
<td>7/9 (78%)</td>
</tr>
<tr>
<td>Severe irritability and/or tremor</td>
<td>32/50 (64%)</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>Arthrogryposis</td>
<td>11/53 (21%)</td>
<td>0/7</td>
</tr>
<tr>
<td>Seizures</td>
<td>10/53 (19%)</td>
<td>0/7</td>
</tr>
</tbody>
</table>
### Congenital Zika syndrome DATA

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>Group 1 MIC</th>
<th>Group 2 NL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcifications</td>
<td>55/57 (96%)</td>
<td>9/9 (100%)</td>
</tr>
<tr>
<td>Cerebellar abnormalities</td>
<td>19/54 (35%)</td>
<td>0/9</td>
</tr>
<tr>
<td>White matter volume loss</td>
<td>57/57 (100%)</td>
<td>9/9 (100%)</td>
</tr>
<tr>
<td>Ventriculomegaly</td>
<td>53/57 (93%)</td>
<td>7/9 (78%)</td>
</tr>
<tr>
<td>Skull collapse (bony cap)</td>
<td>30/57 (53%)</td>
<td>1/9 (11%)</td>
</tr>
<tr>
<td>Brain by MRI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal cortex (PMG)</td>
<td>20/20 (100%)</td>
<td>1/1</td>
</tr>
<tr>
<td>Heterotopia - periventricular</td>
<td>5/18 (28%)</td>
<td>0/1</td>
</tr>
<tr>
<td>Corpus callosum abnormal</td>
<td>19/20 (95%)</td>
<td>1/1</td>
</tr>
<tr>
<td>Brainstem hypoplasia</td>
<td>9/20 (45%)</td>
<td>1/1</td>
</tr>
</tbody>
</table>
Congenital Zika syndrome (N=57)

- **Common (approaching 100%)**
  - Congenital microcephaly, frequently severe (81%)
  - Malformation of cortical development c/w PMG
  - Volume loss causing enlarged extra-axial space, ventriculomegaly or both
  - Punctate intracerebral calcifications that are most numerous in the subcortical white matter, frequent in the basal ganglia and thalami and less common elsewhere
  - Spastic quadriparesis associated with marked irritability and tremors

- **Less common (20-50%)**
  - Retinal and optic nerve abnormalities
  - Hypoplasia of the basal ganglia or cerebellum
  - Fetal brain disruption sequence
  - Arthrogryposis
Aedes in USA