Brazilian Zika virus strain causes birth defects in experimental models

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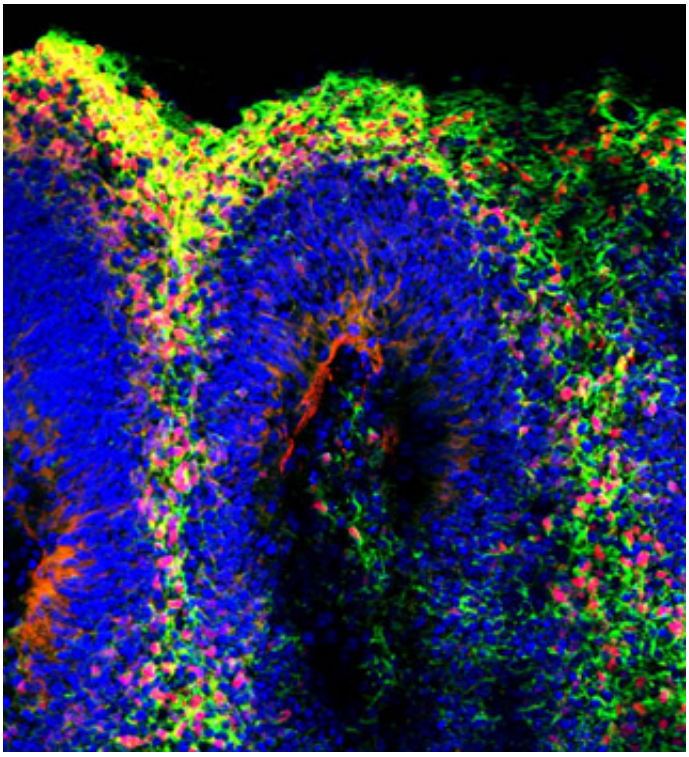


Cross section of a human cerebral organoid shows different structures reminiscent of human embryonic cortical development, such as the proliferative zone in the center, with migrating cells to the surface forming the cortical plate. Cortical progenitor cells are in red, neurons in green and cell nuclei in blue. Credit: UC San Diego Health

Researchers at University of California San Diego School of Medicine, with colleagues in Brazil and Senegal, have described the first "direct

experimental proof" that the Brazilian strain of Zika virus can actually cause severe birth defects. The findings are published in the May 11 online issue of *Nature*.

The team, headed by Alysson R. Muotri, PhD, associate professor in the UC San Diego School of Medicine departments of Pediatrics and Cellular and Molecular Medicine, and co-corresponding senior author Patricia C.B. Beltrao-Braga, PhD, at the University of Sao Paulo, conducted studies in mouse models, human stem cells and in cerebral organoids - miniature brains grown *in vitro*.

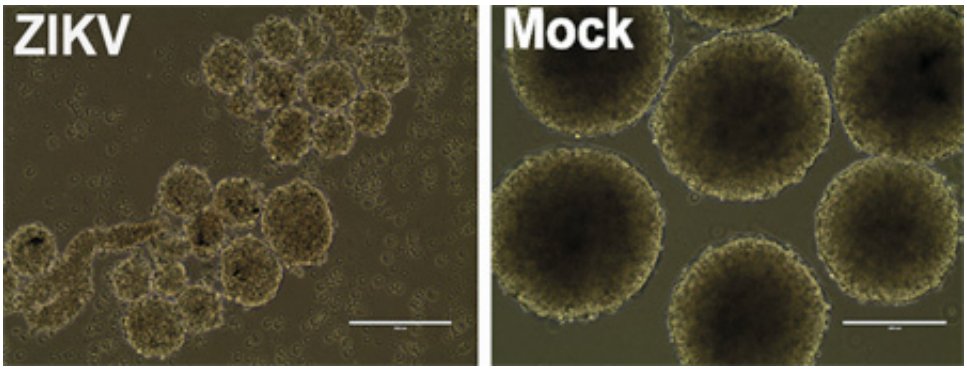
"Rising infection rates of Zika virus in places like Brazil, with a corresponding increase in cases of microcephaly, have powerfully suggested an association, but until now hard evidence has been lacking," said Muotri. "Our findings provide direct experimental proof that the Brazilian Zika virus strain causes severe birth defects - and that the full adverse effect upon health, even beyond microcephaly, is not yet fully understood."

Muotri said the model developed to determine Zika cause-and-effect provides a new tool to assess the effectiveness of potential therapies to counteract the virus during human neurodevelopment.

"Our platform can now be used to understand what is unique about the Brazilian Zika virus and to test drugs to prevent the neurological problems associated with the infection," he said. "Moreover, we now have a robust animal model that will be useful during validation of potential vaccines against the virus."

The Zika virus is not new. It was first described in 1947 in rhesus monkeys in Uganda, but researchers say the twin lineages of Zika - African and Asian - did not cause meaningful infections in humans until 2007, when the Asian strain, carried by *Aedes aegypti* mosquitoes,

caused an epidemic on the Pacific island of Yap. Further outbreaks occurred in New Caledonia and French Polynesia from 2013 to 2015.



Human neurospheres infected with the Brazilian Zika virus after 96 hours. Compared to mock-infected controls, the neurospheres show dramatic cell death with arrested growth, resulting is significantly reduced size. Credit: UC San Diego Health

In 2013, the Asian lineage of Zika reached Brazil and subsequently other countries in South and Central America. In Brazil, the virus has aroused international attention and concern, with infections of pregnant women alarmingly linked to congenital malformations, including microcephaly (an undersized head and brain) in newborns and other severe neurological diseases, such as Guillain-Barre syndrome.

In the *Nature* paper, authors used mouse models to track Brazilian virus infections to birth defects. "This is the first animal model to document Zika-induced birth defects. It shows that the virus can cross the placenta membrane and infect the fetus," said Muotri. Like humans, newborn mouse pups infected via their mothers with the Brazilian Zika virus strain displayed smaller-than-normal heads and stunted body growth. Tissue and genetic analyses revealed other abnormalities, including eye

problems and ongoing cell death.

"The data in mice also suggest that microcephaly is only the tip of the iceberg. The animals have extensive intra-uterine growth arrest, which essentially means poor fetal development in the womb. Media covering the Zika story have focused upon affected babies with small heads because such images are profoundly dramatic, but the true health impact is likely to be more widespread and devastating."

Interestingly, Muotri noted that not all mouse models tested showed a causal effect when infected by the Zika virus. In at least one strain of mice, the Brazilian Zika virus could not cross the placenta of the mother to infect her unborn pups. Muotri said the finding suggests that in mice - and humans - some individuals may be more susceptible to infection than others, possibly due to genetic differences or varying robustness of the immune system response.

The researchers also conducted studies using human pluripotent stem cells to generate cortical progenitor cells that ordinarily would differentiate into neurons forming the brain's cerebral cortex or folded outer layer. Infection of these cortical progenitor cells by the Brazilian viral strain resulted in increased progenitor cell death. The effects of the African virus is not as pronounced, indicating that the mutations of the Brazilian strains made the virus more aggressive in human cells.

Finally, researchers exposed human brain organoids - three-dimensional buds of cells created from pluripotent stem cells that structurally represent specific organs writ small - to the Zika virus. They noted that infection resulted in reduced areas of growth in the organoids and disrupted cortical layers. Again, the Brazilian virus had a more dramatic impact on cortical malformations in these human organoids.

Muotri said they tested the Brazilian Zika virus in organoids derived

from chimpanzees to assess its adaptability compared to the African strain. "The Brazilian virus has a slow replication rate in the chimp organoid compared to the African virus," Muotri said, "which suggests that the Brazilian strain has, somehow, adapted to humans. We are investigating how genetic differences might cause that difference."

More information: *Nature*, DOI: 10.1038/nature18296

Provided by University of California - San Diego