

What to Expect When You're Expecting Zika

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The 2015 emergence of Zika virus (ZIKV) in the Americas brought new attention to this previously obscure virus. Experimental model systems have been instrumental in rapidly advancing our understanding of ZIKV pathogenesis. Here, Lazear looks back on the events leading to the development of the ZIKV mouse model reported in *Cell Host & Microbe*.

My first interaction with Zika virus (ZIKV) was as a handy addition to the virus crossword emerging on the breakroom whiteboard (down from the “z” in “influenza” to the “a” from “Ebola”). Always fond of obscure viruses, I felt pretty clever, though when asked what it was I didn't have much to say beyond “it's a flavivirus,” maybe adding “it's causing outbreaks in the Pacific” (Lazear and Diamond, 2016). As a postdoctoral fellow in Michael Diamond's laboratory at Washington University in St. Louis, my research focused on another flavivirus, West Nile virus (WNV), particularly the roles of different interferon subtypes in restricting viral neuroinvasion and pathogenesis. But after 5 years in the Diamond Laboratory, I was on the job market and looking for open areas of research where I could distinguish myself as a new investigator.

The emergence of new pathogens (and re-emergence of old ones) is a constant theme in arbovirus research, as travel and commerce move infected humans, animals, and arthropod vectors into new areas. Examples in recent decades include the introduction of WNV to North America in 1999; independent introductions of chikungunya virus to Southern Europe in 2007 and to the Western Hemisphere in 2013; and the spread of Schmallenberg virus across Europe since 2011. This is not a new phenomenon (the trans-Atlantic slave trade brought yellow fever virus to the Americas in the 17th century) but modern travel has expedited it, and climate change is likely to expand the geographic areas affected. I wanted to be prepared to study the next emerging arbovirus, and I did not have to look far to see evidence that this approach could pay off: my own postdoctoral mentor had started his laboratory at Wash U in

2001, just as WNV was making its way westward across the United States. Although Mike had planned for his new lab to work on dengue virus, the focus of his postdoctoral training, he was able to adapt this expertise to an emerging flavivirus, quickly becoming a leader in the field. More recently, what had started as a minor research area in the Diamond Laboratory, chikungunya virus, had gained new relevance when this virus was introduced to the Caribbean in 2013 (Diamond, 2015; Racaniello, 2016).

I thought the best candidate for the next epidemic was Usutu virus, a flavivirus similar to WNV. Though not yet a significant cause of human disease, Usutu virus had emerged in Eastern Europe over the past decade and was making its way westward, causing bird die-offs along the way—reminiscent of WNV in North America in the early 2000s. To get started, I requested a sample of Usutu virus from the World Reference Center for Emerging Viruses and Arboviruses, at the University of Texas Medical Branch (UTMB). While I was at it, I also requested some other potential emerging flaviviruses, including Zika virus (ZIKV). This virus was even more obscure than Usutu virus (almost no experimental science published), but it was causing outbreaks in the South Pacific, so had potential to spread further.

Once the viruses arrived in the spring of 2015, I grew stocks and titrated them, tested antibodies for cross-reactivity, and infected mice. At the end of June, Mike went to the National Institutes of Health for a meeting on chikungunya virus but found the coffee break conversations dominated by ZIKV, as Brazilian researchers described a growing outbreak of febrile illness caused by this obscure virus. Learning this, Mike returned to

St. Louis determined to apply his expertise in flavivirus pathogenesis to this emerging public health concern.

On Tues June 30, 2015, at 2:28PM Diamond, Michael wrote:

Helen

Did we ever get the Zika virus from UTMB?

Mike

On Tues June 30, 2015, at 3:34PM Lazear, Helen wrote:

Yep. I have it working in cell culture (virus stocks, FFA), and ASC approval is pending (any updates, Jen?)

There is an outbreak in Brazil now. Importations much more likely now than when it was just in the South Pacific.

Helen

What had been a minor side project (one I had not even bothered to mention in job interviews) became more intense that summer, as it became clear that the ZIKV outbreak was spreading in Brazil.

Aside from the initial description of ZIKV published in 1952, and one paper published in 1971, there was no literature describing ZIKV pathogenesis in animals that we could use as a basis for our studies—certainly nothing using modern inbred mouse lines or transgenic animals. Fortunately, we were able to draw on our extensive experience studying other flaviviruses in mice. Knowing that WNV causes lethal disease in wild-type (C57BL/6) mice but that dengue virus replicates very poorly even in highly immunodeficient mice, I infected both wild-type mice and ones with defective antiviral responses. These included mice that cannot respond to interferon (IFN- α/β)

(*Ifnar1*^{-/-}), mice that lack transcription factors required for IFN- α/β production (*Irf3*^{-/-} \times *Irf5*^{-/-} \times *Irf7*^{-/-}), and mice with defective pattern recognition receptor signaling (*Mavs*^{-/-}), all of which succumb rapidly to WNV infection (Lazear et al., 2013; Suthar et al., 2010). We eventually tested at least 18 different mouse lines at different ages with a variety of ZIKV strains, doses, and inoculation routes. Among weaned mice (3 weeks and older), we only observed morbidity or mortality in lines that lacked an IFN- α/β response (*Ifnar1*^{-/-}, *Ifnar1*^{-/-} \times *Ifngr1*^{-/-}, or *Irf3*^{-/-} \times *Irf5*^{-/-} \times *Irf7*^{-/-}), consistent with what other groups have since reported (Aliota et al., 2016; Dowall et al., 2016; Rossi et al., 2016; Yockey et al., 2016).

While I focused on mouse experiments, a post-baccalaureate student, Derek Platt, and a graduate student, Estefania Fernandez, worked to generate ZIKV reagents. We had not yet been able to obtain any virus isolates from Brazil (a challenge faced by many researchers early in the ZIKV epidemic), but the Centers for Disease Control and Prevention (CDC) had sent us a ZIKV isolate from the South Pacific, thought to be the source of ZIKV introduced to Brazil. Mike and I had agreed that his group would continue generating monoclonal antibodies (a core expertise of the Diamond Laboratory), while I would pursue basic virology and pathogenesis questions as a side project in my new laboratory at the University of North Carolina at Chapel Hill. At this point there was no indication that ZIKV was anything other than the most recent in a series of old-world arboviruses introduced to the Western Hemisphere.

I was 3 weeks into my new position at UNC when the news came of a surge in microcephaly cases in northeastern Brazil, the epicenter of the ZIKV epidemic.

On Wed Nov 18, 2015, at 9:58 AM, Lazear, Helen wrote:

Mike -

What do you think about this suggestion that ZIKV could be associated with microcephaly in Brazil?

http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=32285&lang=en

<http://www.telegraph.co.uk/news/worldnews/southamerica/brazil/12003083/Fears-that-Zika-virus-is-causing-deformities-in-babies-as-it-spreadsacross-South-America.html>

I'm not aware of an association with any other flaviviruses, although there may be associations with some bunyaviruses (especially in livestock). It seems that if it happened with DENV at any reasonable rate, we would know about it. In utero infection with WNV has been studied (by CDC) and was associated with some neurologic abnormalities, but specifically not microcephaly. I wonder if drugs taken to treat symptoms might be a more likely culprit? It's a huge increase in number of cases.

Helen

On Wed Nov 18, 2015, at 11:49AM Diamond, Mike wrote:

Yes, I heard this from the Brazilian colleagues

Not sure, but could be possible. not seen with other flavis to my knowledge

m

Early on, I was skeptical of a possible association between ZIKV and microcephaly, a congenital defect in which the fetal brain fails to develop, because other flaviviruses are not generally associated with birth defects and ZIKV seemed only to cause a self-limited febrile illness. However, a substantial body of evidence now demonstrates that ZIKV infection during pregnancy can result in Congenital Zika Syndrome, a collection of developmental disorders that includes microcephaly (Coyne and Lazear, 2016).

Public interest in ZIKV surged in early 2016, with the spread of heartbreaking images of Brazilian infants with tiny misshapen heads. Realizing that ZIKV was a much more significant human pathogen than we had initially appreciated, we felt a new sense of urgency to publish our mouse infection data, since small animal models are incredibly valuable for understanding mechanisms of viral pathogenesis as well as evaluating potential vaccines and therapeutics. However, in my new laboratory I did not yet have pipettes or a freezer, let alone

the large colony of knockout mice that would be necessary to complete this paper. I am grateful to members of the Diamond Laboratory, including Jonathan Miner, Amber Smith, and Jennifer Govero, who expended tremendous effort to complete additional experiments in record time. They infected hundreds of immunodeficient mice with different ZIKV strains, watching and waiting to see whether any additional lines would succumb to infection. They detected high viral titers in tissues such as the brain and testes that we now think are important for ZIKV pathogenesis. They also demonstrated that ZIKV can replicate in wild-type mice administered an IFNAR1-blocking monoclonal antibody, resulting in high viremia (though not lethality). Subsequent work in the Diamond laboratory by Jonathan, Jennifer, and others demonstrated that this increase in viremia is enough to elicit key ZIKV disease presentations including transplacental transmission, ophthalmological pathology, and damage to the testes with a concomitant decrease in male fertility (Govero et al., 2016; Miner et al., 2016a, 2016b). Although there are inherent limitations to studying viral pathogenesis in the absence of IFN- α/β signaling, having small animal models that replicate severe manifestations of ZIKV infection is important for developing interventions and for understanding the basic biology of ZIKV. Thanks to the effort and dedication of everyone involved, we were able to submit our paper to *Cell Host and Microbe* in March 2016 (Lazear et al., 2016). The urgency surrounding ZIKV research was reflected in the paper's rapid turnaround: the revised version was accepted 10 days later. Unbeknownst to us, Rossi and colleagues had already submitted a report of ZIKV infection in *Ifnar1*^{-/-} mice to the *American Journal of Tropical Medicine and Hygiene* (Rossi et al., 2016); when we first saw their publication (on the day ours was accepted), we knew this was the beginning of a deluge of ZIKV publications.

When abstracts were due for the 2016 American Society for Virology meeting, ours was the only one about ZIKV submitted by the February 1 deadline. However, the field was moving so quickly that by May, two late-breaking workshops were added for the June meeting, including 20 oral presentations and 9 posters. Among the many advances published in



Figure 1. The Author and Her 10-Day-Old Son Attending the 2016 Meeting of the American Society for Virology

2016 were multiple ZIKV cryo-EM and crystal structures; demonstrations of ZIKV infection of and damage to neuronal cells and organoids; studies in mice modeling congenital and sexual transmission; investigations of antiviral immunity in the placenta; and non-human primate studies that replicated key features of ZIKV disease in humans. While the field moved from case reports to prospective studies, a causal role for ZIKV in the development of fetal microcephaly was demonstrated, there was a growing appreciation for the range of outcomes that comprise Congenital Zika Syndrome, evidence that ZIKV could be sexually transmitted, and multiple ZIKV vaccine candidates were developed and began the early stages of clinical trials.

Although many questions remain about the pathologic mechanisms of ZIKV, and researchers will pursue these for years to come, the pace of ZIKV research in 2016 truly was extraordinary. One factor in the rapid progress of this research is that ZIKV can be studied under biosafety level 2 (BSL-2) containment ([Centers for Disease Control and Prevention, 2009](#)). This is an important difference between ZIKV and other emerging viruses, such as WNV, chikungunya, and MERS coronavirus, which must be handled under BSL-3 containment, or Ebola and Nipah viruses, which require BSL-4 containment. Policies that require higher levels of biocontainment “out of an abundance of caution” may underestimate the benefits that come when diverse researchers can easily study an emerging virus.

Although the CDC lists ZIKV as a BSL-2 agent, individual laboratories and institu-

tions are free to require higher levels of containment or additional safety precautions; these range from none, to additional signage, to supplementary personal protective equipment, to BSL-3 containment. As we and other groups at UNC began research with ZIKV, our institutional biosafety committee (IBC) was chiefly concerned with additional precautions to protect pregnant women from ZIKV exposure. Though surely a concern at other institutions as well, in January 2016 we were in the unusual position of devising policies to protect not just pregnant women in general, but also one very specific pregnant woman, since I myself was expecting my third child.

Early protocols submitted to our IBC by other labs simply stated that pregnant women would not work with ZIKV, a policy that would be unenforceable while also creating a disincentive to disclosure. My opinions about this were informed by my experience as a postdoctoral fellow, when I was pregnant with my first child. When I told Mike that I was pregnant, he asked that I stop doing BSL-3 sharps work, due to the risk of a needlestick exposure. Nearly all of my experiments involved infecting mice with WNV and harvesting their tissues, so it was no small request when Mike asked another postdoctoral fellow in the lab to do these experiments for me. I was shocked and felt that my project was being taken away, and it was embarrassing to be burdening someone else in the lab with my work. Ironically, this postdoc was herself pregnant, though she had not yet revealed this to Mike or to the rest of the laboratory. She continued to do her experiments and mine, and my experience discouraged her from disclosing her pregnancy any earlier than necessary. Two years later, when I was pregnant with my second child, we decided not to change any of my experimental procedures. My experience left me sensitive to the complex motivations involved in these decisions, particularly for a graduate student or postdoctoral fellow with an intellectual and emotional investment in a project. Now that I am a Principal Investigator, I have come to appreciate that the risks I might knowingly take upon myself exceed those that I would accept for my trainees. Carefully considering the risks involved and designing thoughtful policies to address them is important to avoid penalizing

women and to enable their informed decision making.

When considering the proper policies to adopt for ZIKV research, our IBC’s questions ranged from the risks of a splash exposure to whether I would be nimble enough to catch an escaped mouse. We ultimately agreed on a system of enhanced information and consent for all laboratory workers, additional warning signs, and accommodations for women who declined to work with ZIKV. We decided that cell culture work was low-risk under routine BSL-2 containment and that the main exposure risk was needlesticks. Therefore, pregnant women would be advised to avoid sharps work with ZIKV. These precautions were reasonable and enabled my new laboratory to begin work on most of our ZIKV research projects but left me unable to do the mouse infections that were my primary expertise; I owe many thanks to Ken Plante, then a postdoctoral fellow in Mark Heise’s laboratory, who stepped up to infect mice with ZIKV while I could not. The irony, given the measures we took to find someone from another lab to assist me with sharps work, was that I had spent the earliest part of my pregnancy injecting ZIKV into as many mice as possible (at least 157 by my records), frantically trying to generate data before leaving Wash U. Of course, none of us had heard of microcephaly then. As the evidence for Congenital Zika Syndrome grew, along with indications that the first trimester may be an especially vulnerable time for infection, I was thankful that I had no reason to think that I had had an exposure during those early experiments. My healthy baby was born in June 2016 and 10 days later he was the youngest attendee (I presume) at the American Society for Virology meeting at Virginia Tech (see [Figure 1](#)), where I convened one of the late-breaking sessions on ZIKV and presented our work developing a mouse model of ZIKV pathogenesis.

I do not think you need to be pregnant, a woman, or a parent to have your heart ache for the children born from ZIKV-affected pregnancies and the families who care for them. However, I do think that my experience has made me especially aware of the privilege I had to avoid exposure to ZIKV while I was pregnant, which sadly is not an option for many women in ZIKV endemic areas. This

highlights the need for a vaccine to prevent ZIKV infection, as well as better data about the factors that mediate transplacental transmission and the long-term prognosis for children exposed to ZIKV in utero, along with access to comprehensive family planning services (including contraception and abortion) so that women confronted with ZIKV exposure can make informed decisions.

Despite the tremendous advances in our understanding of ZIKV during 2016, many questions remain about the basic virology and pathogenic mechanisms of this virus, as well as its epidemiology and clinical presentations. There are also technical hurdles to overcome for the development of serological diagnostics and an effective vaccine. The importance of these questions has attracted researchers with diverse expertise; studying Zika virus has provided an exciting opportunity to work with colleagues in neuroscience, developmental biology, and maternal-fetal medicine, as well as other virologists and immunologists. I have had the great fortune to be starting my new lab at UNC among incredibly supportive neighbors and colleagues who have made it possible for my laboratory to move our ZIKV research forward, even before I was able to get all of our equipment and assays up and running. My laboratory has broad and ongoing interests in ZIKV pathogenesis, including the impact of genetic changes between

historical and contemporary ZIKV strains on pathogenesis; the innate immune mechanisms that control viral invasion across anatomical barriers; and the impact of host genetics and prior flavivirus immunity on susceptibility to ZIKV disease. Mouse infection systems, and particularly the model we reported in our *Cell Host & Microbe* paper in 2016, are proving to be key resources for addressing these and other questions in ZIKV research.

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REFERENCES

- Aliota, M.T., Caine, E.A., Walker, E.C., Larkin, K.E., Camacho, E., and Osorio, J.E. (2016). *PLoS Negl. Trop. Dis.* *10*, e0004682.
- Centers for Disease Control and Prevention (2009). *Biosafety in Microbiological and Biomedical Laboratories, Fifth Edition* (U.S. Department of Health and Human Services).
- Coyne, C.B., and Lazear, H.M. (2016). *Nat. Rev. Microbiol.* *14*, 707–715.
- Diamond, M.S. (2015). *PLoS Pathog.* *11*, e1005182.
- Dowall, S.D., Graham, V.A., Rayner, E., Atkinson, B., Hall, G., Watson, R.J., Bosworth, A., Bonney, L.C., Kitchen, S., and Hewson, R. (2016). *PLoS Negl. Trop. Dis.* *10*, e0004658.
- Govero, J., Esakky, P., Scheaffer, S.M., Fernandez, E., Drury, A., Platt, D.J., Gorman, M.J., Richner, J.M., Caine, E.A., Salazar, V., et al. (2016). *Nature* *540*, 438–442.
- Lazear, H.M., and Diamond, M.S. (2016). *J. Virol.* *90*, 4864–4875.
- Lazear, H.M., Lancaster, A., Wilkins, C., Suthar, M.S., Huang, A., Vick, S.C., Clepper, L., Thackray, L., Brassil, M.M., Virgin, H.W., et al. (2013). *PLoS Pathog.* *9*, e1003118.
- Lazear, H.M., Govero, J., Smith, A.M., Platt, D.J., Fernandez, E., Miner, J.J., and Diamond, M.S. (2016). *Cell Host Microbe* *19*, 720–730.
- Miner, J.J., Cao, B., Govero, J., Smith, A.M., Fernandez, E., Cabrera, O.H., Garber, C., Noll, M., Klein, R.S., Noguchi, K.K., et al. (2016a). *Cell* *165*, 1081–1091.
- Miner, J.J., Sene, A., Richner, J.M., Smith, A.M., Santeford, A., Ban, N., Weger-Lucarelli, J., Manzella, F., Rückert, C., Govero, J., et al. (2016b). *Cell Rep.* *16*, 3208–3218.
- Racaniello, V. (2016). TWiV 414: Zika in the guys with Diamond. In *This Week in Virology*. <http://www.microbe.tv/twiv/twiv-414/>.
- Rossi, S.L., Tesh, R.B., Azar, S.R., Muruato, A.E., Hanley, K.A., Auguste, A.J., Langsjoen, R.M., Paessler, S., Vasilakis, N., and Weaver, S.C. (2016). *Am. J. Trop. Med. Hyg.* *94*, 1362–1369.
- Suthar, M.S., Ma, D.Y., Thomas, S., Lund, J.M., Zhang, N., Daffis, S., Rudensky, A.Y., Bevan, M.J., Clark, E.A., Kaja, M.K., et al. (2010). *PLoS Pathog.* *6*, e1000757.
- Yockey, L.J., Varela, L., Rakib, T., Khoury-Hanold, W., Fink, S.L., Stutz, B., Sziget-Buck, K., Van den Pol, A., Lindenbach, B.D., Horvath, T.L., and Iwasaki, A. (2016). *Cell* *166*, 1247–1256.e4.