2016

Mechanisms of Zika virus infection and neuropathogenesis

David Olagnier
McGill University

Michela Muscolini
Istituto Pasteur-Fondazione Cenci Bolognetti

Carolyn B. Coyne
University of Pittsburgh

Michael S. Diamond
Washington University School of Medicine in St. Louis

John Hiscott
Istituto Pasteur-Fondazione Cenci Bolognetti

DNA AND CELL BIOLOGY
Volume 35, Number 8, 2016

Pp. 367–372
DOI: 10.1089/dna.2016.3404

DNACB BIT

Mechanisms of Zika Virus Infection and Neuropathogenesis

David Olagnier,[1,*] Michela Muscolini,[2,*] Carolyn B. Coyne,[3] Michael S. Diamond,[4–6] and John Hiscott[2]

A spotlight has been focused on the mosquito-borne Zika virus (ZIKV) because of its epidemic outbreak in Brazil and Latin America, as well as the severe neurological manifestations of microcephaly and Guillain–Barré syndrome associated with infection. In this review, we discuss the recent literature on ZIKV-host interactions, including new mechanistic insight concerning the basis of ZIKV-induced neuropathogenesis.

Introduction

Zika virus (ZIKV) was originally isolated from an infected monkey in the Zika forest of Uganda in 1947 (Dick and Haddow, 1952). For decades, transmission of this arbovirus to humans via the bite of the *Aedes aegypti* mosquito was thought to cause a mild and self-limiting febrile syndrome, with most (~80%) infections being clinically inapparent. This perception has changed with epidemic outbreaks in Yap Island in 2007, French Polynesia in 2013, and in Brazil and other Latin American countries in 2015–2016. The number of ZIKV infections has exploded and, with it, a causal association of microcephaly and other congenital malformations in fetuses and newborns of ZIKV-infected mothers (Brasil *et al.*, 2016a), as well as Guillain–Barré syndrome (Brasil *et al.*, 2016b; Cao-Lormeau *et al.*, 2016; Watrin *et al.*, 2016) and meningoencephalitis (Carteaux *et al.*, 2016).

New modes of transmission, including *in utero* and sexual transmission, as well as the presence of ZIKV in breast milk, have been observed. ZIKV is now an emerging pathogen that poses an imminent global threat (Brasil *et al.*, 2016a; Broutet *et al.*, 2016; Calvet *et al.*, 2016; Dupont-Rouzeyrol *et al.*, 2016; Hills *et al.*, 2016; Sarno *et al.*, 2016). In this review, we summarize the most recent observations on the biology, tropism, and neuropathogenesis associated with ZIKV.

Virus Structure

ZIKV belongs to the *Flavivirus* genus of the *Flaviviridae* family, which includes other human pathogens such as dengue, West Nile, yellow fever, tick-borne encephalitis, and Japanese encephalitis viruses. ZIKV is a 50 nm spherical virion that comprised three structural proteins (capsid [C], premembrane/membrane [prM/M], and envelope [E]), a lipid envelope, and a 10.7 kb capped RNA of positive polarity. The E protein is a class II viral membrane fusion protein that has an elongated three-domain (domains [D] I–III) structure (Dai *et al.*, 2016). E protein directs several critical steps of the viral life cycle, including engagement with cellular attachment and entry factors, membrane fusion, and virus assembly. Recent high-resolution cryoelectron microscopy of intact ZIKV virions was reported (Kostyuchenko *et al.*, 2016; Sirohi *et al.*, 2016). The ZIKV structure is similar to other flavivirus structures, except for ~10 amino acids that surround an N-linked glycosylation site in E-DI. The carbohydrate moiety associated with this residue has been speculated to contribute to an attachment site for the virus to host cells. As this region varies among flaviviruses, it could influence the unique aspects of ZIKV transmission and disease (Sirohi *et al.*, 2016).

ZIKV is believed to form only one serotype (Tappe *et al.*, 2015) with two main ZIKV lineages, African and Asian (Haddow *et al.*, 2012; Faye *et al.*, 2014). Wang *et al.* (2016) recently completed detailed phylogenetic and genetic analyses, as well as targeted structural modeling on all known full-length open reading frames of ZIKV. This study showed that ZIKV strains in the recent human outbreak evolved from the Asian lineage, and that all human strains identified in the 2015–2016 epidemic appear closely related to the H/PF/2013 strain isolated in French Polynesia in 2013 (Wang *et al.*, 2016). In a separate report, phylogenetic and molecular clock analyses performed on seven Brazilian ZIKV strains suggested a single introduction of ZIKV into the Americas more than 1 year before the first cases were detected in Brazil (Faria *et al.*, 2016). However, another study identified a slight separation between Haitian and Brazilian strains, indicating that ZIKV was circulating in Haiti as early as middle 2013, before the first reported cases in Brazil (Lednicky *et al.*, 2016). Sequencing analysis also identified amino changes in the outbreak lineage; however, no common substitutions across the three currently available genomes from microcephaly cases were apparent (Faria *et al.*, 2016).

[1]Lady Davis Institute, Jewish General Hospital, McGill University, Montreal, Canada.
[2]Istituto Pasteur–Fondazione Cenci Bolognetti, Rome, Italy.
[3]Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania.
Departments of [4]Medicine, [5]Molecular Microbiology, and [6]Pathology and Immunology, The Center for Human Immunology and Immunotherapy Programs, Washington University School of Medicine, St. Louis, Missouri.
*These two authors contributed equally to this review.

Host–Virus Interactions

Host–virus interactions that shape ZIKV infection remain poorly characterized. Hamel *et al.* (2015) were the first to show that human dermal fibroblasts, epidermal keratinocytes, and immature dendritic cells all were permissive to a ZIKV isolate from French Polynesia. TLR3 was identified as the initial immune receptor involved in the sensing of ZIKV infection in human fibroblasts leading to type I and type II interferon (IFN) responses (Hamel *et al.*, 2015). ZIKV also interacted with DC-SIGN to initiate infection of immature Mo-DCs, whereas members of the TIM and TAM family of phosphatidylserine receptors possibly serve as receptors or attachment factors for other cells; in cutaneous fibroblasts and epidermal keratinocytes lacking expression of DC-SIGN, the TAM receptor AXL facilitated ZIKV entry (Hamel *et al.*, 2015).

Nowakowski *et al.* (2016) examined the receptor repertoire of human radial glia cells in the fetal brain involved in ZIKV attachment and entry during neurogenesis. Distinct flavivirus entry receptor genes, including AXL receptor, were enriched in radial glia cells, astrocytes, endothelial cells, and microglia, suggesting that these cell populations may be particularly vulnerable to ZIKV infection in the developing brain (Fig. 1). Both single-cell RNA-sequencing and immunohistochemistry analyses confirmed high levels of expression of the AXL receptor on radial glia cells (Nowakowski *et al.*, 2016); in contrast, other entry receptor

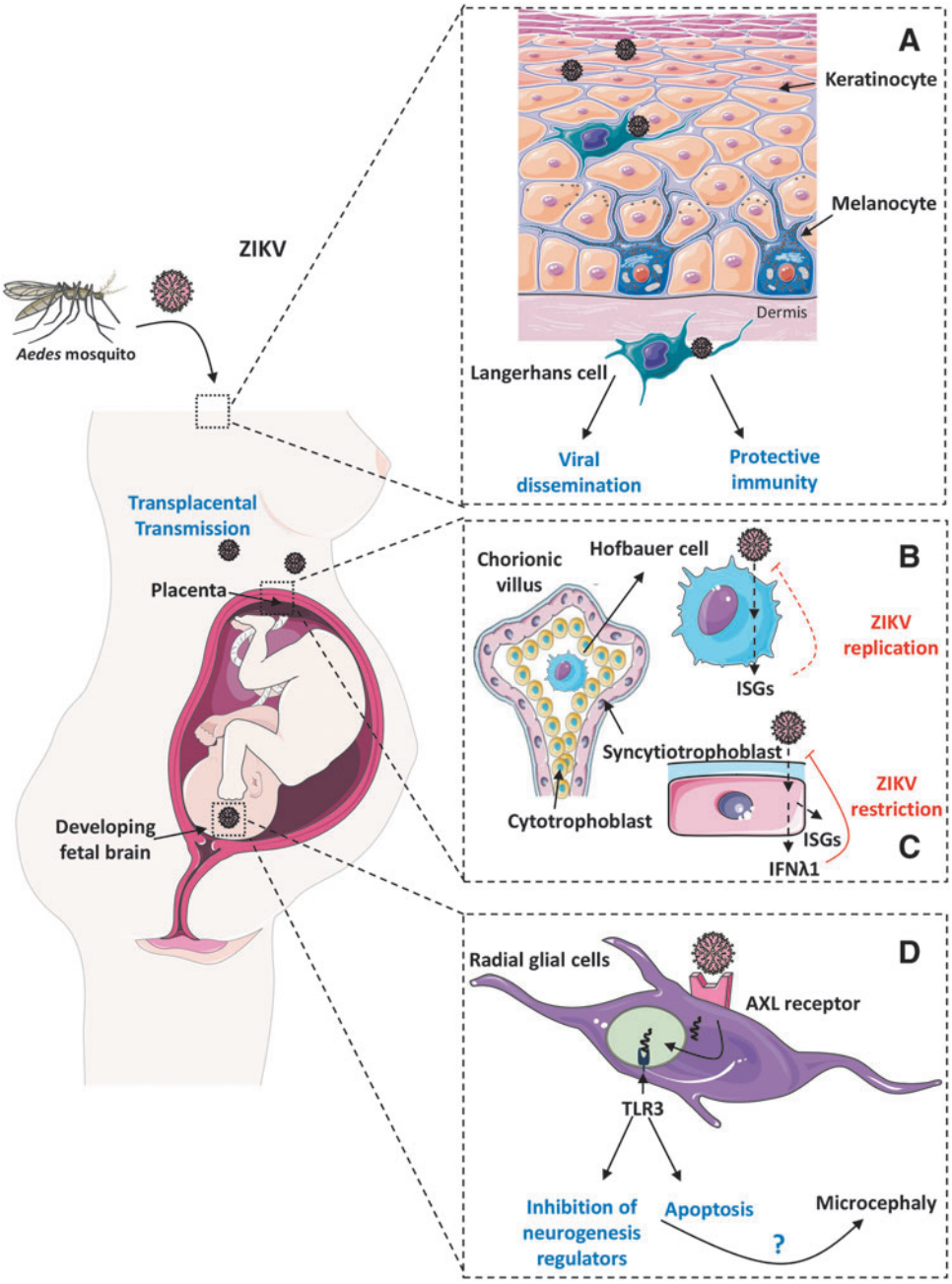

FIG. 1. ZIKV infection mechanisms and neuropathogenesis. ZIKV is transmitted via the bite of an infected *Aedes* mosquito. **(A)** Both dermal fibroblasts and epidermal keratinocytes are targets of ZIKV infection, potentially transmitting infection to dermal dendritic cells (Langerhans cell), thereby facilitating ZIKV dissemination. **(B)** Transplacental delivery of ZIKV to fetus may occur by infection of cytotrophoblasts or transmigration of infected primary human placental macrophages (Hofbauer cells), suggesting a novel mechanism for intrauterine transmission. On ZIKV infection, Hofbauer placental macrophages secrete type I IFN and upregulate ISGs, although cells remain permissive to ZIKV replication. **(C)** At late stages of pregnancy, the production of IFNλ1 and ISGs by placental syncytiotrophoblasts may exert protective effects inhibiting ZIKV infection. **(D)** ZIKV directly targets neural progenitor cells during the first trimester of fetal brain development. The TAM receptor AXL may have an important role in ZIKV entry within neural cells. ZIKV-dependent activation of TLR3-mediated immune responses leads to dysregulation of genes involved in neuronal development and apoptosis, resulting in severe damage to the embryonic brain, including microcephaly. ISGs, interferon stimulated genes; ZIKV, Zika virus. Figure illustrated by David Olagnier using the Servier Medical Art library (www.servier.fr/smart/banque-dimages-powerpoint).

candidates such as TYRO3 and DC-SIGN showed more limited expression in the fetal brain.

While the characterization of AXL expression within cells of the central nervous system (CNS) is a first step toward defining ZIKV tropism, further studies are required to confirm whether AXL determines ZIKV tropism in neuronal cells or in murine models *in vivo*, beyond its reported ability to enhance attachment/entry *in vitro* in skin fibroblasts.

Efficient antagonism of the innate antiviral response is crucial for flaviviruses that rely on viremia to maintain the vector–host cycles. By analogy with dengue virus (DENV), ZIKV infection resulted in the degradation of the IFN-regulated transcriptional activator STAT2 in Vero cells, thus inhibiting IFN-mediated induction of interferon stimulated genes (ISGs) (Grant *et al.*, 2016). Ectopic expression of the nonstructural protein NS5 of ZIKV in HEK 293T cells was sufficient to target STAT2 for proteasomal degradation. DENV NS5 previously was demonstrated to bind STAT2 and requires the E3 ubiquitin ligase UBR4 to promote STAT2 degradation (Ashour *et al.*, 2009; Morrison *et al.*, 2013). Like DENV, ZIKV NS5 interacted with STAT2, although independently of UBR4 (Grant *et al.*, 2016). Interestingly, STAT2 levels were unaffected in ZIKV-infected primary mouse embryonic fibroblasts and ZIKV replication was impaired, suggesting that ZIKV IFN antagonism may be a species-restricted mechanism (Grant *et al.*, 2016).

Infection of Neural Progenitor Cells

ZIKV tropism extends to human neural progenitor cells (hNPCs). Human-induced pluripotent stem cells differentiate into forebrain-specific hNPCs and were infected efficiently by the ZIKV prototype strain MR-766. ZIKV infection also increased cell death and dysregulated cell cycle progression, resulting in attenuated growth of cultured stem cell-derived human cortical progenitors (Tang *et al.*, 2016); global gene expression analysis of infected hNPCs further demonstrated transcriptional dysregulation of cell cycle-related pathways. These results establish hNPCs, a constitutive population of the developing embryonic brain, as a target of ZIKV infection and injury, providing a first clue to the neuropathology linked with microcephaly and other CNS abnormalities (Tang *et al.*, 2016).

Other studies have examined the effects of ZIKV infection in human neural stem cells grown as neurospheres and brain organoids (Dang *et al.*, 2016; Garcez *et al.*, 2016). Neurospheres represent the very early stages of neurogenesis, whereas brain organoid cultures recapitulate the early cellular and molecular events of first-trimester fetal neocortex, including gene expression and cortical layering (Lancaster *et al.*, 2013; Camp *et al.*, 2015). ZIKV-infected hNPCs generated very few neurospheres, and those displayed morphological abnormalities and cell detachment, compared to the hundreds of neurospheres detected under control conditions. Apoptotic nuclei, as well as smooth membrane structures, were observed in all ZIKV-infected neurospheres (Garcez *et al.*, 2016). These models mimic some features of first-trimester brain development and suggest that ZIKV infection during this time window may damage the developing brain.

Human embryonic stem cell-derived organoid cultures were also used to investigate how ZIKV infection may lead to microcephaly during the first trimester of fetal brain development. Infection with the prototypical ZIKV MR-766 strain caused impaired development and severely inhibited growth of cerebral organoids by activating TLR3-mediated innate immune responses (Fig. 1). Interestingly, TLR3 inhibition reduced the phenotypic effects of ZIKV infection, and a transcriptome profile of developing organoids revealed dysregulation of a network of genes related to neuronal development and apoptotic pathways following TLR3 stimulation (Dang *et al.*, 2016). This study offers mechanistic insight into the basis of microcephaly in ZIKV-infected newborns.

Murine Models of ZIKV Pathogenesis

The establishment of murine models, and knowledge of their strengths and limitations, will be extremely important in the study of ZIKV pathogenesis, maternal-fetal transmission, and development of vaccines and therapeutics (Becker, 2016).

Because ZIKV is sensitive to the antiviral effects of mouse type I and type II IFNs (Hamel *et al.*, 2015), the impact of ZIKV infection has been explored in IFN-deficient murine models (Aliota *et al.*, 2016; Dowall *et al.*, 2016; Lazear *et al.*, 2016; Miner *et al.*, 2016; Rossi *et al.*, 2016; Zmurko *et al.*, 2016). Using different knockout models and contemporary as well as historical ZIKV strains, Lazear *et al.* (2016) demonstrated that 4- to 6-week-old $Irf3^{-/-}Irf5^{-/-}Irf7^{-/-}$ triple knockout mice, which produce little type I IFN, or mice lacking the IFN receptor ($Ifnar1^{-/-}$) developed neurological disease and succumbed to ZIKV infection, whereas single $Irf3^{-/-}$, $Irf5^{-/-}$, or $Mavs^{-/-}$ knockout mice exhibited no overt illness. $Ifnar1^{-/-}$ mice sustained high viral loads in the brain and spinal cord, consistent with neurodevelopmental defects. The highest viral loads were observed in the testes of $Ifnar1^{-/-}$ mice, an observation that is relevant to sexual transmission of ZIKV (Lazear *et al.*, 2016). Also, a low-passage Cambodian isolate of ZIKV was shown to cause disease and mortality in $Ifnar^{-/-}$ A129 mice in an age-dependent manner (Rossi *et al.*, 2016). Mice infected at the youngest age (3 weeks) showed early viremia at day 2; ZIKV was detected in the brain at day 3 and by day 6, signs of neurologic disease were observed. These animals succumbed to illness by day 7, whereas in older mice (11 weeks), signs of illness, viremia, and weight loss were detected, but the animals began to recover on day 8 (Rossi *et al.*, 2016). In another study, AG129 mice deficient in both IFN-α/β and γ receptors, succumbed to ZIKV infection within 7–8 days; rapid virus dissemination was observed in visceral organs and the brain, but was associated with severe pathology only in the brain and muscle (Aliota *et al.*, 2016). Collectively, these studies indicate that murine models of ZIKV pathogenesis, although in immunocompromised strains, recapitulate some of the features of human neurological disease.

Importantly, ZIKV infection of the $Ifnar1^{-/-}$ mouse model during pregnancy recapitulated features of placental tropism and fetal demise associated with *in utero* transmission of ZIKV (Miner *et al.*, 2016). $Ifnar1^{-/-}$ female mice were crossed with wild-type males and then inoculated with ZIKV on embryonic day 6.5 or 7.5. ZIKV infected the pregnant dams, resulting in damage to the placental barrier and infection of the developing fetus, accompanied by trophoblast infection, placental insufficiency, and intrauterine growth restriction (Miner *et al.*, 2016).

Using contemporary ZIKV strains, vertical transmission of ZIKV and a marked effect on fetal brain development were characterized (Cugola *et al.*, 2016; Li *et al.*, 2016; Wu *et al.*, 2016). A Brazilian ZIKV strain ($ZIKV^{BR}$) crossed the placenta and caused microcephaly by targeting cortical progenitor cells, causing cell death by apoptosis and autophagy. Moreover, ZIKV infection impaired fetal neurodevelopment and caused intrauterine growth restriction (Cugola *et al.*, 2016). Infection with a contemporary Asian ZIKV strain (SZ01) led to cell cycle arrest, apoptosis, and inhibition of NPC differentiation, thus disrupting neural progenitor development *in vivo* and resulting in cortical thinning and microcephaly in mice (Li *et al.*, 2016). Infection of pregnant mice with ZIKV SZ01 led to the infection of radial glia cells, with fetal mice exhibiting a reduced cavity of lateral ventricles and a decrease in surface areas of the cerebral cortex (Wu *et al.*, 2016).

Together, these models provide a link between ZIKV infection in neural cells and microcephaly, with potential for further exploration of the underlying mechanisms.

Vertical Transmission and Transplacental Routes of ZIKV Infection

The strong association between ZIKV infection in pregnant women with the development of catastrophic neurodevelopmental outcomes suggests that ZIKV gains access to the intrauterine cavity directly to affect fetal development.

During mammalian pregnancy, the placenta acts as the sole physical and immunological barrier between the maternal and fetal compartments. Primary human trophoblasts (PHTs) consist of both cytotrophoblasts and syncytiotrophoblasts that are the key barrier cells, with syncytiotrophoblasts directly bathed in maternal blood (Fig. 1). PHTs from term placentas are refractory to ZIKV infection due to their constitutive release of antiviral IFNλ1 (Bayer *et al.*, 2016). In addition, medium from uninfected PHT cells protected nonplacental cells from ZIKV infection, and PHT cells themselves expressed high levels of ISGs, indicating that IFNλ1 functions in both paracrine and autocrine manners to protect trophoblast and nontrophoblast cells (Bayer *et al.*, 2016).

Trophoblast cell lines cultured from choriocarcinoma explants and first-trimester human villous explants, respectively, were susceptible to ZIKV infection (Miner *et al.*, 2016). Conversely, placentas nearer to term, which form a more fully developed placental barrier, exhibited greater resistance to infection. To infect syncytiotrophoblasts, ZIKV likely must evade the restriction imparted by trophoblast-derived IFNλ1 and other trophoblast-specific antiviral factors (Delorme-Axford *et al.*, 2013) and/or gain access to the fetal compartment using alternative strategies to cross the placental barrier, at least in the second half of pregnancy, the gestational period most represented by PHT cells. These pathways might include nontrophoblast infectious routes, such as infection via immune cells or transcytosis of virions complexed to maternal immunoglobulins via the neonatal Fc receptor (FcRn).

Human placental macrophages (Hofbauer cells [HCs]) recently were described as permissive to ZIKV infection (Quicke *et al.*, 2016). These innate immune cells may represent key target cells in the placenta targeted by the virus once it penetrates the trophoblast layer. On infection, HCs produced IFN-α, proinflammatory cytokines, and activated antiviral gene expression, although cell death was not significantly induced (Fig. 1). Human placental cytotrophoblasts also supported ZIKV replication, but with delayed replication kinetics and ISG expression (Quicke *et al.*, 2016). Thus, studies to date point to a role for ZIKV replication within cytotrophoblasts and HCs as a possible mechanism to facilitate vertical transmission. However, as syncytiotrophoblasts are refractory to ZIKV infection (Bayer *et al.*, 2016), the mechanism(s) by which ZIKV accesses the underlying cytotrophoblast layer and HCs remains unknown. It is also important to note that the human placenta undergoes significant morphological changes between the first and second half of pregnancy, the most notable of which are the lack of maternal blood contact with the syncytiotrophoblast layer in the first trimester of pregnancy and the significant reduction in the cytotrophoblast cellular layer as pregnancy progresses. Thus, the mechanisms by which ZIKV is transmitted vertically may be distinct at different gestational ages.

Summary and Perspectives

ZIKV infection of human embryonic NPCs provides a potential mechanism for teratogenicity of ZIKV in the developing brain (Dang *et al.*, 2016; Garcez *et al.*, 2016; Tang *et al.*, 2016). These observations are consistent with studies in mice demonstrating that high ZIKV loads in both brain and spinal cord are associated with severe neuropathology (Aliota *et al.*, 2016; Lazear *et al.*, 2016; Rossi *et al.*, 2016) and impairment of fetal brain development (Cugola *et al.*, 2016; Li *et al.*, 2016; Wu *et al.*, 2016). The latest *in vivo* studies establish a direct causal link with microcephaly and other abnormalities in the developing brain.

Despite a protective role of type III IFNλ1 produced by syncytiotrophoblasts of term placentas (Bayer *et al.*, 2016), fetal infection by ZIKV can occur by a transplacental route in early pregnancy, resulting in infection of fetal brain, placental damage, and fetal abortion (Cugola *et al.*, 2016; Li *et al.*, 2016; Miner *et al.*, 2016). However, studies also suggest markedly different susceptibilities and/or distinct mechanisms of pathogenesis at specific times during pregnancy (Bayer *et al.*, 2016; Miner *et al.*, 2016; Quicke *et al.*, 2016). It is possible that viremia in infected mothers, coupled with blood-borne maternal transmission to the developing fetus, establishes a niche for virus growth that would then facilitate neurological teratogenic effects. Once the virus breaches the barrier presented by the trophoblasts, HCs may become productively infected, thus facilitating vertical transmission (Quicke *et al.*, 2016).

As ZIKV research increasingly focuses on mechanisms of neuropathogenesis, lessons learned from other flaviviruses will undoubtedly facilitate a detailed mechanistic understanding of host–virus interactions, as well as contribute to the development of novel therapeutics (Olagnier *et al.*, 2016). Critically important questions about ZIKV pathogenesis have been addressed within a short frame of time, but further work is urgently required to determine factors that impact ZIKV infection, *in utero* transmission, and neuropathogenic mechanisms (Fig. 1).

Acknowledgments

This research was supported by grants from the NIH (AI108861), the Italian Association for Cancer Research, and

the Pasteur GPF Vaccinology program to J.H.; by grants from the Burroughs Wellcome Fund and NIH (R01 AI073755) to M.S.D.; and from the Burroughs Wellcome Fund and NIH (R01 AI081759 and HD075665) to C.B.C.; D.O. was supported by a Peter Quinlan McGill postdoctoral fellowship. The authors acknowledge all the contributions in the field that could not be included in the review.

Disclosure Statement

No competing financial interests exist.

References

Aliota, M.T., Caine, E.A., Walker, E.C., Larkin, K.E., Camacho, E., and Osorio, J.E. (2016). Characterization of lethal Zika virus infection in AG129 mice. PLoS Negl Trop Dis **10,** e0004682.

Ashour, J., Laurent-Rolle, M., Shi, P.Y., and Garcia-Sastre A. (2009). NS5 of dengue virus mediates STAT2 binding and degradation. J Virol **83,** 5408–5418.

Bayer, A., Lennemann, N.J., Ouyang, Y., Bramley, J.C., Morosky, S., Marques, E.T., Jr., *et al.* (2016). Type III interferons produced by human placental trophoblasts confer protection against Zika virus infection. Cell Host Microbe **19,** 705–712.

Becker, R. (2016). Missing link: animal models to study whether Zika causes birth defects. Nat Med **22,** 225–227.

Brasil, P., Pereira, J.P., Jr., Raja Gabaglia, C., Damasceno, L., Wakimoto, M., Ribeiro Nogueira, R.M., *et al.* (2016a). Zika virus infection in pregnant women in Rio de Janeiro—Preliminary report. N Engl J Med [Epub ahead of print]; DOI: 10.1056/NEJMoa1602412.

Brasil, P., Sequeira, P.C., Freitas, A.D., Zogbi, H.E., Calvet, G.A., de Souza, R.V., *et al.* (2016b). Guillain-Barre syndrome associated with Zika virus infection. Lancet **387,** 1482.

Broutet, N., Krauer, F., Riesen, M., Khalakdina, A., Almiron, M., Aldighieri, S., *et al.* (2016). Zika virus as a cause of neurologic disorders. N Engl J Med **374,** 1506–1509.

Calvet, G., Aguiar, R.S., Melo, A.S., Sampaio, S.A., de Filippis, I., Fabri, A., *et al.* (2016). Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis [Epub ahead of print]; DOI: 10.1016/S1473-3099(16)00095-5.

Camp, J.G., Badsha, F., Florio, M., Kanton, S., Gerber, T., Wilsch-Brauninger, M., *et al.* (2015). Human cerebral organoids recapitulate gene expression programs of fetal neocortex development. Proc Natl Acad Sci U S A **112,** 15672–15677.

Cao-Lormeau, V.M., Blake, A., Mons, S., Lastere, S., Roche, C., Vanhomwegen, J., *et al.* (2016). Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet **387,** 1531–1539.

Carteaux, G., Maquart, M., Bedet, A., Contou, D., Brugieres, P., Fourati, S., *et al.* (2016). Zika virus associated with meningoencephalitis. N Engl J Med **374,** 1595–1596.

Cugola, F.R., Fernandes, I.R., Russo, F.B., Freitas, B.C., Dias, J.L.M., Guimarães, K.P., *et al.* (2016). The Brazilian Zika virus strain causes birth defects in experimental models. Nature **534,** 267–271.

Dai, L., Song, J., Lu, X., Deng, Y.Q., Musyoki, A.M., Cheng, H., *et al.* (2016). Structures of the Zika virus envelope protein and its complex with a flavivirus broadly protective antibody. Cell Host Microbe **19,** 696–704.

Dang, J., Tiwari, S.K., Lichinchi, G., Qin, Y., Patil, V.S., Eroshkin, A.M., *et al.* (2016). Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3. Cell Stem Cell [Epub ahead of print]; DOI: 10.1016/j.stem.2016.04.014.

Delorme-Axford, E., Donker, R.B., Mouillet, J.F., Chu, T., Bayer, A., Ouyang, Y., *et al.* (2013). Human placental trophoblasts confer viral resistance to recipient cells. Proc Natl Acad Sci U S A **110,** 12048–12053.

Dick, G.W., and Haddow, A.J. (1952). Uganda S virus; a hitherto unrecorded virus isolated from mosquitoes in Uganda. I. Isolation and pathogenicity. Trans R Soc Trop Med Hyg **46,** 600–618.

Dowall, S.D., Graham, V.A., Rayner, E., Atkinson, B., Hall, G., Watson, R.J., *et al.* (2016). A susceptible mouse model for Zika virus infection. PLoS Negl Trop Dis **10,** e0004658.

Dupont-Rouzeyrol, M., Biron, A., O'Connor, O., Huguon, E., and Descloux, E. (2016). Infectious Zika viral particles in breastmilk. Lancet **387,** 1051.

Faria, N.R., Azevedo Rdo, S., Kraemer, M.U., Souza, R., Cunha, M.S., Hill, S.C., *et al.* (2016). Zika virus in the Americas: early epidemiological and genetic findings. Science **352,** 345–349.

Faye, O., Freire, C.C., Iamarino, A., Faye, O., de Oliveira, J.V., Diallo, M., *et al.* (2014). Molecular evolution of Zika virus during its emergence in the 20(th) century. PLoS Negl Trop Dis **8,** e2636.

Garcez, P.P., Loiola, E.C., Madeiro da Costa, R., Higa, L.M., Trindade, P., Delvecchio, R., *et al.* (2016). Zika virus impairs growth in human neurospheres and brain organoids. Science **352,** 816–818.

Grant, A., Ponia, S.S., Tripathi, S., Balasubramaniam, V., Miorin, L., Sourisseau, M., *et al.* (2016). Zika virus targets human STAT2 to inhibit Type I Interferon signaling. Cell Host Microbe **19,** 882–890.

Haddow, A.D., Schuh, A.J., Yasuda, C.Y., Kasper, M.R., Heang, V., Huy, R., *et al.* (2012). Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS Negl Trop Dis **6,** e1477.

Hamel, R., Dejarnac, O., Wichit, S., Ekchariyawat, P., Neyret, A., Luplertlop, N., *et al.* (2015). Biology of Zika virus infection in human skin cells. J Virol **89,** 8880–8896.

Hills, S.L., Russell, K., Hennessey, M., Williams, C., Oster, A.M., Fischer, M., *et al.* (2016). Transmission of Zika virus through sexual contact with travelers to areas of ongoing transmission—continental United States, 2016. MMWR Morb Mortal Wkly Rep **65,** 215–216.

Kostyuchenko, V.A., Lim, E.X., Zhang, S., Fibriansah, G., Ng, T.S., Ooi, J.S., *et al.* (2016). Structure of the thermally stable Zika virus. Nature **533,** 425–428.

Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., *et al.* (2013). Cerebral organoids model human brain development and microcephaly. Nature **501,** 373–379.

Lazear, H.M., Govero, J., Smith, A.M., Platt, D.J., Fernandez, E., Miner, J.J., *et al.* (2016). A mouse model of Zika virus pathogenesis. Cell Host Microbe **19,** 720–730.

Lednicky, J., Beau De Rochars, V.M., El Badry, M., Loeb, J., Telisma, T., Chavannes, S., *et al.* (2016). Zika virus outbreak in Haiti in 2014: molecular and clinical data. PLoS Negl Trop Dis **10,** e0004687.

Li, C., Xu, D., Ye, Q., Hong, S., Jiang, Y., Liu, X., *et al.* (2016). Zika virus disrupts neural progenitor development and leads to microcephaly in mice. Cell Stem Cell [Epub ahead of print]; DOI: 10.1016/j.stem.2016.04.017.

Miner, J.J., Cao, B., Govero, J., Smith, A.M., Fernandez, E., Cabrera, O.H., *et al.* (2016). Zika virus infection during

pregnancy in mice causes placental damage and fetal demise. Cell **165,** 1081–1091.

Morrison, J., Laurent-Rolle, M., Maestre, A.M., Rajsbaum, R., Pisanelli, G., Simon, V., *et al.* (2013). Dengue virus co-opts UBR4 to degrade STAT2 and antagonize type I interferon signaling. PLoS Pathog **9,** e1003265.

Nowakowski, T.J., Pollen, A.A., Di Lullo, E., Sandoval-Espinosa, C., Bershteyn, M., and Kriegstein, A.R. (2016). Expression analysis highlights AXL as a candidate Zika virus entry receptor in neural stem cells. Cell Stem Cell **18,** 591–596.

Olagnier, D., Amatore, D., Castiello, L., Ferrari, M., Palermo, E., Diamond, M.S., *et al.* (2016). Dengue virus immunopathogenesis: lessons applicable to the emergence of Zika virus. J Mol Biol [Epub ahead of print]; DOI: 10.1016/j.jmb.2016.04.024.

Quicke Kendra, M., Bowen James, R., Johnson Erica, L., McDonald Circe, E., Ma, H., O'Neal Justin, T., *et al.* (2016). Zika virus infects human placental macrophages. Cell Host Microbe [Epub ahead of print]; DOI: 10.1016/j.chom.2016.05.015.

Rossi, S.L., Tesh, R.B., Azar, S.R., Muruato, A.E., Hanley, K.A., Auguste, A.J., *et al.* (2016). Characterization of a novel murine model to study Zika virus. Am J Trop Med Hyg **94,** 1362–1369.

Sarno, M., Sacramento, G.A., Khouri, R., do Rosario, M.S., Costa, F., Archanjo, G., *et al.* (2016). Zika virus infection and stillbirths: a case of hydrops fetalis, hydranencephaly and fetal demise. PLoS Negl Trop Dis **10,** e0004517.

Sirohi, D., Chen, Z., Sun, L., Klose, T., Pierson, T.C., Rossmann, M.G., *et al.* (2016). The 3.8 A resolution cryo-EM structure of Zika virus. Science **352,** 467–470.

Tang, H., Hammack, C., Ogden, S.C., Wen, Z., Qian, X., Li, Y., *et al.* (2016). Zika Virus infects human cortical neural progenitors and attenuates their growth. Cell Stem Cell **18,** 587–590.

Tappe, D., Nachtigall, S., Kapaun, A., Schnitzler, P., Gunther, S., and Schmidt-Chanasit, J. (2015). Acute Zika virus infection after travel to Malaysian Borneo, September 2014. Emerg Infect Dis **21,** 911–913.

Wang, L., Valderramos, S.G., Wu, A., Ouyang, S., Li, C., Brasil, P., *et al.* (2016). From mosquitos to humans: genetic evolution of Zika virus. Cell Host Microbe **19,** 561–565.

Watrin, L., Ghawche, F., Larre, P., Neau, J.P., Mathis, S., and Fournier, E. (2016). Guillain-Barre Syndrome (42 Cases) occurring during a Zika virus outbreak in French Polynesia. Medicine **95,** e3257.

Wu, K.Y., Zuo, G.L., Li, X.F., Ye, Q., Deng, Y.Q., Huang, X.Y., *et al.* (2016). Vertical transmission of Zika virus targeting the radial glial cells affects cortex development of offspring mice. Cell Res **26,** 645–654.

Zmurko, J., Marques, R.E., Schols, D., Verbeken, E., Kaptein, S.J., and Neyts, J. (2016). The viral polymerase inhibitor 7-deaza-2′-C-methyladenosine is a potent inhibitor of in vitro Zika virus replication and delays disease progression in a robust mouse infection model. PLoS Negl Trop Dis **10,** e0004695.

Address correspondence to:
John Hiscott, PhD
Istituto Pasteur–Fondazione Cenci Bolognetti
Viale Regina Elena 291
Rome 00161
Italy

E-mail: john.hiscott@istitutopasteur.it

Received for publication June 6, 2016; accepted June 6, 2016.