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Review

Probing Molecular Insights of Zika Virus-Host Interactions

Ina Lee[1], Sandra Bos1[5], Ge Li[1], Shusheng Wang[1], Gilles Gadea[5], Philippe Despres[5], Richard Y. Zhao[1-4]*

Department of Pathology[1], Department of Microbiology and Immunology[2], Institute of Global Health[3], and Institute of Human Virology[4], University of Maryland School of Medicine, Baltimore, MD 21201, USA; [5]Université de la Réunion, INSERM U1187, CNRS UMR 9192, IRD UMR 249, Unité Mixte Processus Infectieux en Milieu Insulaire Tropical, Plateforme Technologique CYROI, 94791 Sainte Clotilde, La Réunion, France

*, Correspondence: rzhao@som.umaryland.edu (R.Y.Z); Tel: +1 410 706 6301; E-mail:

Abstract: The recent Zika virus (ZIKV) outbreak in Americas surprised all of us because of its rapid spread and association with neurologic disorders including fetal microcephaly, brain and ocular anomalies and Guillain-Barré syndrome. In responding to this global health outcry, unprecedented and world-wide efforts are taking place to study the ZIKV etiology. Much have been learned about this virus in the areas of epidemiology, clinical manifestation, viral sequences and protein structures, as well as effects of ZIKV infection on fetal brain development and microcephaly. However, the molecular mechanism underlying ZIKV-mediated neurologic disorders remains elusive. Some critical questions include: 1) what type of virologic changes has taken place that increased the viral virulence? 2) which ZIKV protein(s) is responsible for the enhanced viral pathogenicity? And 3) how the newly adapted and pathogenic ZIKV strains alter their interactions with host cells leading to neurologic disorders? The goal of this review is to explore the molecular insights into the ZIKV-host interactions with special focuses on host cell receptor usage for viral entry, host cellular and immune antiviral responses, ZIKV counteraction and ZIKV-induced cytopathic effects. Our hope with this literature review is to inspire additional studies focusing on molecular studies of ZIKV-host Interactions.

Keywords: Zika virus; ZIKV-host interactions; viral pathogenesis; cell surface receptors; antiviral responses; viral counteraction; cytopathic effects; microcephaly; ZIKV-associated neurologic disorders

1. Introduction

The Zika virus (ZIKV): an emerging public health threat. The 2015 Zika virus (ZIKV) epidemic in the Americas surprised the world because of its rapid global spread and the findings that it associates with various neurologic disorders including microcephaly in newborns and Guillain-Barré syndrome (GBS) in adults. ZIKV was thought to be a mild virus that had little or no threat to humans. Through studies of this new ZIKV pandemic, we now learned that ZIKV is a rather severe human pathogen that, besides fetal microcephaly and GBS, it also induces various other congenital neurologic and ocular disorders [1,2]. So it begs the question as what has transformed the benign ZIKV over the past 50 years to the pathogenic ZIKV we see today.

The goal of this article is to review the current literature and explore the molecular insights into the ZIKV-host interactions. Different from many other review articles, here we are taking a different approach by focusing on molecular aspects of ZIKV-host interactions. We will summarize what we have learned about host cellular and immune responses to ZIKV infection. Conversely, ZIKV evasion to host's antiviral

defense and ZIKV-induced cytopathic effects will be described. The molecular mechanisms underlying those ZIKV-host interactions and their potential impacts on ZIKV-induced fetal microcephaly or other neurologic disorders will be discussed.

The Zika virus. ZIKV belongs to the flavivirus family that includes a number of highly pathogenic viruses such as Dengue Virus (DENV), Japanese Encephalitis Virus (JEV) and West Nile Virus (WNV). The structure of ZIKV is similar to those of other flaviviruses. The nucleocapsid is approximately 25-30 nm in diameter, surrounded by a host membrane-derived lipid bilayer that contains envelope (E) and membrane (M) proteins. The virion is approximately 40-65 nm in diameter, with surface projections that measure roughly 5-10 nm [3], leading an overall average size of 45-75 nm. The surface proteins are arranged in an icosohedral-like symmetry [4] Like its flaviviral siblings, ZIKV is an enveloped virus that contains a positive sense single-stranded RNA [ssRNA(+)] viral genome of approximately 10.7 kilobases (kb). The genomic RNA is flanked by two terminal flanking non-coding regions (NCR), *i.e.*, the 5' NCR (107 nt) and the 3' NCR (428 nt) [5]. The ZIKV genome encodes a single large open reading frame encoding a polyprotein of about 3,300 amino acids, which is processed co- and post-translationally by viral and host proteases (PRs) to produce a total of 14 immature proteins, mature proteins and small peptides [6]. However, only a total of ten mature viral proteins, *i.e.*, three structural proteins (C, M and E) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins are found in infectious viral particles [3,6,7]. A 2K signal peptide, which lies between NS4A and NS4B, plays a regulatory role in viral RNA synthesis and viral morphogenesis in other flaviviruses [8,9]. Among the structural proteins, the mature capsid (C) protein is produced by cleavage of the anchor-C (anaC) protein by a viral PR (anaC→C), which in turn triggers the cleavage of the precursor membrane (prM) protein by the host signalase. As a result, a mature membrane (M) protein and a Pr protein are produced (PrM→M + Pr) [8,10]. In the case of DENV, noninfectious and immature viral particles contain prM that forms a heterodimer with the E protein [11]. The transition of prM to M by Furin cleavage results in mature and infectious particles [12,13]. The E protein, composing the majority of the virion surface, is involved in binding to the surface of host cells and triggering subsequent membrane fusion and endocytosis [5]. Post-translation processing of the non-structural protein region produces four viral enzymes, *i.e.*, PR, helicase, methyl-transferase and RNA-dependent RNA polymerase (RdRP). ZIKV PR consists of the two protein components NS2B and NS3. A fully active ZIKV PR consists of two protein components, namely the N-terminal domain of NS3 (NS3Pro) and a membrane-associated NS2B cofactor [14,15]. The NS3Pro is responsible for proteolytic processing the viral polyprotein; whereas the NS2B cofactor is required for enhancing enzymatic activity and substrate specificity. The C-terminal domain of NS3 protein produces ZIKV helicase, which plays a critical role in NTP-dependent RNA unwinding and translocation during viral

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replication [16]. The methyl-transferase and RdRP are generated from the N-terminal and C-terminal of NS5, respectively. NS1, NS3, and NS5 are large proteins that are highly-conserved [3]. NS2A, NS2B, NS4A, and NS4B proteins are smaller, hydrophobic proteins [3]. The 3' NCR forms a loop structure that may play a role in translation, RNA packaging, cyclization, genome stabilization and recognition [5]. The 5' NCR allows translation *via* a methylated nucleotide cap or a genome-linked protein [4]. In addition, ZIKV produces abundant non-coding subgenomic flavivirus RNA (sfRNA) from the 3'UTR in infected cells, which may play a role in the viral life cycle and viral subversion to innate immunity [17].

The life cycle of ZIKV and human transmission. The reproductive cycle of ZIKV is like those of other flaviviruses. The Zika viral particle starts to bind to the host cell surface receptors *via* the E protein, which triggers the fusion between viral and endosomal membranes that leads to clathrin-dependent endocytosis. Next, the virus membrane fuses with the endosomal membrane in a pH-dependent manner, and the ssRNA genome of the virus is released into the cytoplasm of the host cell. The positive-sense viral genomic ssRNA is translated into a polyprotein that is subsequently cleaved to form structural and non-structural proteins. Viral replication takes place at intracellular membrane-associated compartments located at the surface of endoplasmic reticulum (ER), resulting in a dsRNA genome synthesized from the genomic ssRNA(+) by viral RNA-dependent polymerase. The dsRNA genome is subsequently transcribed and replicated, resulting in additional viral mRNAs/ssRNA(+) genomes. Assembly of new virions occurs within the ER, where the virions are transported through the trans-Golgi network (TGN) and to be excreted into the intracellular space where the new virions can move on to a new infection life cycle[4].

ZIKV is an arbovirus that is predominately transmitted to mammalian hosts by using mosquitoes as vectors [18]. A variety of mosquitoes from the genus *Aedes* include *A. africanus, A. aegypti, A. vitattus, A. furcifer, A. apicoargenteus,* and *A. luteocephalus* [18,19]. The incubation time for ZIKV in mosquito vectors is approximated 10 days [19]. Blood contact *via* blood transfusion or sexual contact is another route of ZIKV infection [20-22]. Consistent with this notion, human testis has been found to be a reservoir for ZIKV [19,23-25]. In addition, ZIKV could be transmitted vertically from mother to child *via* placenta-fetal transmission [26,27]. This has become a main route for the development of fetal microcephaly [28,29].

A brief history. The ZIKV was first isolated in 1947 from caged monkeys in the Zika forest of Uganda, Africa. A ZIKV strain MR766 ($ZIKV_{MR766}$) from that isolation was established and has been used for research since [30]. Therefore, the $ZIKV_{MR766}$ is often referred as the historical or ancestral ZIKV strain. Initial characterization of $ZIKV_{MR766}$ showed that it is highly neurotropic in mice and no virus has been

recovered from tissues other than the brains of infected mice [31]. That report further showed that mice at all ages were susceptible to $ZIKV_{MR766}$ by intracerebral inoculations. In contrast, cotton-rats, Guinea pigs and rabbits showed no sign of $ZIKV_{MR766}$ infection by using the same intracerebral inoculations. Monkeys showed either mild fever (pyrexia) or no signs of infection. Interestingly, mice younger than 2 weeks were highly susceptible to intraperitoneal inoculation, mice of 2 weeks. In contrast, mice older than 2 weeks can rarely be infected by the intraperitoneal route [31], suggesting established blood-brain barrier in the older mice may have prevented ZIKV from access to the brain.

In a different study, the effect of ZIKV infection on the central nervous system (CNS) of mice was examined by using intracerebral inoculation [32]. Histologic H&E staining showed that ZIKV infects Ammon's horn (hippocampus proper) area of the 7 day old mouse brain. Detailed examination suggested that ZIKV infected a layer of pear-shaped cells known as pyriform cells of Ammon's horn and induced hyperchromatic debris in those cells, suggesting possible DNA or chromosomal aberration. In addition, ZIKV induced gross enlargement (hypertrophy) of astroglial cells of Ammon's horn but it had little effect on microglial cells in the same area [32]. Ultra-structural studies by electron microscopy (EM) further revealed that ZIKV replicates exclusively in the ER compartment of astroglial cells and neurons, an indication of membrane-associated viral replication [32].

Those early findings in the mouse model suggest that 1) ZIKV is a neurotropic virus with preference to embryonic brains [30,31], 2) ZIKV specifically infect astroglial cells, pyriform cells in Ammon's horn, and 3) ZIKV primarily replicates in the ER network [32]. At the cellular level, ZIKV appeared to induce gross cell enlargement, chromosomal or DNA aberration and increase of mitochondrial dysfunction [32]. Although early data showed that ZIKV was pathogenic to mice, there was no indication it was pathogenic to humans [31]. Therefore, some types of virologic changes most likely have taken placed during the viral evolution in the past 50 years leading ZIKV infection to humans.

The first ZIKV infection in human was documented in 1952 [30], and the virus was subsequently isolated from human hosts in Nigeria in 1968 [19]. Since then, multiple studies have confirmed the presence of ZIKV antibodies in human sera in a number of countries in Africa and Asia [19]. However, no serve diseases were clearly linked to those infections. In the recorded history, ZIKV infection appears to migrate from Africa toward the east globally. A number of outbreaks have taken place over the past 50 years including several minor outbreaks in 1977-1978 in Pakistan, Malaysia and Indonesia. Two major outbreaks were documented in Yap Island of Micronesia in 2007 and in French Polynesia, New Caledonia, the Cook Islands, and Easter Island in 2013 and 2014 [22,33]. The affected individuals in those outbreaks were in the order of hundreds to thousands. However, in the most recent outbreaks, transmission of ZIKV infection had been reported in 85 countries, territories, or subnational areas with an estimate of over 1.5 million affected individuals according to the World Health Organization (WHO). Brazil was the

most affected country, with an estimate of 440,000 to 1.3 million cases reported through December of 2015.

Although human ZIKV infection is mostly self-limiting, cases of neurologic manifestations such as GBS were apparently increasingly during the ZIKV outbreaks in French Polynesia and Brazil [28,34]. The number of microcephaly in newborns was also increased dramatically, which for the first time indicated a possible link between ZIKV and fetal malformations [28]. More than 4,700 suspected cases of microcephaly were initially reported from mid-2015 through January 2016 [35]. In the meantime, an unprecedented and world-wide effort took place to uncover this mystery. By March of 2016, the causal relationship between microcephaly in newborn and the ZIKV infection was first established [28]. By April of 2016, a total of 3,530 newborns with confirmed microcephaly were reported. In the same year, WHO declared international public health emergency. In-depth research now shows that ZIKV infection also associate with a number of other congenial and ocular diseases [1,2].

What we have learned from the ZIKV pandemic. We have learned a great deal about the ZIKV and its etiology through the above described studies. The knowledge we gained so far is that fetal microcephaly can indeed be caused by the ZIKV infection [28,36,37]. Furthermore, those circulating and pathogenic ZIKV strains are most likely derived from the Asian lineage rather than African lineage [38,39]. Specifically, the Asian lineage is likely evolved from the African lineage through viral gene mutations by the adaptation of higher cytopathicity that led to enhanced viral pathogenicity. The Asian lineage appears to have different viral replication kinetics, narrower host cell target range and slower cell killing effect than the African lineage [40]. Therefore, the newly adapted pathogenic ZIKV could become 1) preferentially infect certain human tissues or cells especially neural progenitor cells (NPCs) in brain, or 2) acquire higher virulence through individual or combined effects of ZIKV gene mutations [41-43]. The antibody-dependent enhancement (ADE) may also contribute to the acquired virulence [44]. This could occur in individuals who have previously been exposed to other flaviviruses generating antibodies that are partially reactive to the ZIKV. Neutralizing antibodies present in those individuals could, instead of neutralize ZIKV, actually augment ZIKV infection and lead to increased infectivity [44]. This situation is possible because there were precedents that pre-exposure of ZIKV infection enhances DENV-2 infection *in vitro* [45] and in monkey [46]. A reciprocal ADE effect could certainly be conceivable [47,48]. However, ADE is less likely to be the predominant mode of enhanced ZIKV pathogenicity since ZIKV causes fetal microcephaly often in the absence of antibody response to other flaviviruses. The third possibility is that the ZIKV-induced microcephaly could be caused by pathogenic ZIKVs that are intrinsic to all ZIKVs, *i.e.*, the microcephaly observed today is simply the reflection of the advanced technology in disease monitoring and diagnosis. Thus, it could actually be a public oversight due to the lack of sensitive detection methods in the past.

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This possibility may not totally be far-fetching because the very first ZIKV isolate, $ZIKV_{MR766}$, also induces microcephaly in animal and human models [37,49,50]. The original $ZIKV_{MR766}$ appears to be more virulent and causes more severe brain damage than the Asian lineage in embryonic mouse brains [51]. Through studies of using human fetal tissues, organoids or animal models, microcephaly or microcephaly-like phenotypes have been described and caused by ZIKV strains through the entire epidemic spectrum (**Table 1**), *e.g.*, MR766 [37,49,50], FSS13025 (Cambodia/2010} [49], PF/2013/KD507 (French Polynesia/2013) [52], SZ01 [49] and various epidemic Brazilian strains (ZIKV-BR/2015) [28,53].

In short, even though we have learned great deals about the ZIKV etiology, much remains to be learned. The questions include 1) what type of virologic changes has taken place that resulted in increased viral pathogenicity? 2) which ZIKV protein(s) is responsible for the enhanced viral pathogenicity? and 3) how the newly adapted ZIKV strains alter their interactions with host cells leading to those neurologic disorders? In particular, the specific mechanisms underlying the molecular actions of ZIKV-mediated neurologic disorders such as microcephaly and other neurologic disorders remain elusive.

Viral pathogenicity is normally referred to the process by which a viral infection leads to disease. The level of viral pathogenicity is often determined by the target of organ, tissue and cells (cell tropism), the level and persistence of viral replication in host cells, and degree of viral damages to host cells often known as the cytopathic effects (CPEs). Since ZIKV remains neurotropic and replicates in brain-specific neuronal cells since the discovery of ZIKV [30], viral factors other than the viral cell tropism are more likely contributing to the increased viral pathogenicity leading to congenital fetal microcephaly and other neurologic disorders. Further, those pathologic enhancement is less likely attributed solely by one or few viral gene mutations, but rather an overall and combined effects of ZIKV-host interactions. Overall, it could be the changing balance between the ZIKV-host interactions that led to 1) favorable and persistent ZIKV viral replication in host cells such as hNPCs, and 2) increased and lasting CPEs that ultimately contribute to those observed fetal development and neurologic disorders. In the following sections, we will discuss the molecular aspects of ZIKV-host interactions, which include 1) selection of host target cells and cell surface receptors during viral entry, 2) the host cellular and immune responses to ZIKV infections, 3) the counteracting effects of ZIKV to host antiviral responses, and 4) ZIKV-induced cytopathic effects including restriction of host cell growth, cell cycle dysregulation and cell death/apoptosis, which are all known contributing to fetal brain development and other neurologic impairments [36,50,54].

2. Cellular Targets and Viral Entry

Cellular targets. ZIKV primarily infects NPCs in embryonic brains [36,54,55]. In brain, it also infect astroglial, microglial cells and to less extent neurons [32,36]. In addition, ZIKV infects other tissues

including skin, testis, and placenta, and other cell types including skin cells such as dermal fibroblasts and epidermal keratinocytes (**Table 2**). Because ZIKV is an arbovirus, its transmission is predominately transmitted through skin by mosquitoes such as *A. africanus* and *A. aegypti* [18,19]. Consistent with this route of transmission, immature dendritic and dendritic cells are conducive to ZIKV infection [56-58]. ZIKV can also be transmitted through sexual contacts [20-22]. Infected Sertoli cells in human testis are now considered as a reservoir for ZIKV [23-25]. Several placenta-specific cells were prone to ZIKV infection including Hofbauer cells, trophoblasts and placental endothelial cells, supporting an important role of placenta in transmitting ZIKV *via* blood to embryonic brains [29,59,60]. In line with this idea that passing the blood-brain barrier might be required to transmit the virus to the brain compartment [31], ZIKV persistently infects primary human brain microvascular endothelial cells (hBMECs) or established cell lines [61]. Interestingly, a hepatoma cell line Huh-7 appears to be highly permissive to ZIKV infection. However, liver has not yet been documented to be the target organ of ZIKV [62].

The cellular receptors for ZIKV entry. Flaviviruses enter host cells by endocytosis, which is initiated when the virus particles interact with cell surface receptors. The cell surface receptors bind to the infectious viral particles and direct them to the endocytic pathway. Several cell surface receptors facilitate ZIKV viral entry. They include the tyrosine-protein kinase receptor AXL, Tyro3, DC-SIGN, and TIM-1 [56]. AXL and Tyro3 are part of the TAM receptor tyrosine kinase family that normally bind to Gas6 and Pros1 ligands to regulate an array of cellular activities including cell adhesion, migration, proliferation and survival, as well as regulation of inflammatory cytokine release, playing pivotal roles in innate immunity [63]. DC-SIGN is an innate immune receptor present on the surface of both macrophages and dendritic cells (DCs). It recognizes a broad range of pathogen-derived ligands and mediates antigen uptake and signaling [64]. The TIM-1 receptor, also known as HAVcr-1 (Hepatitis A virus cellular receptor 1), plays an important role in host response to viral infection.

Even though all of those cell surface receptors (AXL, Tyro3, DC-SIGN and TIM-1) participate in ZIKV viral entry, they are not unique to ZIKV infection. For example, AXL, Tyro3 and DC-SIGN are used by Lassa virus [65]. The TIM-1 receptor mediates infections of the deadly Ebola virus [66]. In fact, both TAM and TIM families of phosphatidylserine receptors also mediate viral entry of other flaviviruses such as DENV [67] and WNV [68]. For instance, in the case of DENV, TIM receptors facilitate viral entry by directly interacting with virion-associated phosphatidylserine; whereas TAM-mediated infection relies on indirect viral recognition, in which the TAM ligand Gas6 acts as a bridging molecule by binding to phosphatidylserine within the viral particle [67]. A review of this subject can be found in [69].

Involvement of AXL, Tyro3, DC-SIGN, and, to a lesser extent, TIM-1 were initially described by Hamel *et al.* when they studied ZIKV entry in skin cells [56]. AXL was subsequently shown to be a prime target

receptor for ZIKV viral entry in variety of cell types including human endothelial cells (hECs) [54], neural stem cells [70], microglia and astrocytes [71], and oligodendrocyte precursor cells [72]. Examination of the AXL expression levels of diverse cell types suggests that AXL is highly expressed on the surface of human radial glial cells, astrocytes, hECs, oligodendrocyte precursor cells and microglia in developing human cortex as well as in progenitor cells in the developing retina [70,72]. Other ZIKV permissive and non-neuronal human cells including placental cells, explants-cytotrophoblasts, endothelial cells, fibroblasts, and Hofbauer cells in chorionic villi as well as amniotic epithelial cells and trophoblast progenitors in amniochorionic membranes express AXL, Tyro3, and/or TIM1 that are likely their viral entry cofactors [73].

The susceptibility of human ECs to ZIKV positively correlates with the cell surface levels of AXL [54]. Gain- and loss-of-function tests revealed that AXL is required for ZIKV entry at a post-binding step, and small-molecule inhibitors of the AXL kinase significantly reduced ZIKV infection of hECs [54]. In human microglia and astrocytes of the developing brain, like DENV, AXL-mediated ZIKV entry requires the AXL ligand Gas6 to serve as a bridge linking ZIKV particles to glial cells. Following binding, ZIKV is internalized through clathrin-mediated endocytosis and is transported to Rab5+ endosomes to establish productive infection. Downregulation of AXL by an AXL inhibitor R428 or an AXL decoy receptor MYD1 significantly reduced the ZIKV infection, suggesting the AXL receptor might be the primary receptor but it may not be the only receptor that is required for ZIKV infection [71]. Genetic knockdown of AXL in a glial cell line nearly abolished ZIKV infection [72].

Interestingly, genetic ablation of the AXL receptor by CRISPR/CAS9 did not protect human neural progenitor cells (NPCs) and cerebral organoids from ZIKV Infection [74]. In particular, genetic ablation of AXL has no effect on ZIKV entry or ZIKV-mediated cell death in human induced pluripotent stem cell (iPSC)-derived NPCs or cerebral organoids. It is not yet clear what contributes to the observed discrepancy between this and other studies. One possibility is that ZIKV may use different cell surface receptors iPSC-derived NPCs [74]. For example, TIM-1 plays a more prominent role than AXL in placental cells [73]. Duramycin, a peptide that binds phosphatidylethanolamine in enveloped virions precludes TIM1 binding, reduced ZIKV infection in placental cells and explants. In a mouse study, comparison of AXL knock-out mice with homozygous or heterozygous mice showed no significant differences in ZIKV

viral replication and clinical manifestation, suggesting AXL is dispensable for ZIKV infection in those mice [75].

3. Cellular and Immune Responses to ZIKV Infection

Inflammation is one of the first line responses of the cellular immune system to viral infection, which is typically ignited by releasing cytokines including chemokines (**Table 3**). ZIKV triggers various *host cell pro-inflammatory responses* [56,57,76,77]. For example, ZIKV stimulates CD8+ T cell-mediated polyfunctional immune responses to induce NFkB-mediated production of cytokines such as IL-1β, IL-6, MIP1α as well as chemokines including IP10 and RANTES [76,78] (**Figure 1**). These ZIKV-induced T cell immune responses are antiviral because introduction of CD8+ T cells isolated from ZIKV-infected mice into naïve mice prior to ZIKV infection enhances viral clearance. Conversely, depletion of CD8+ T cells from infected animals compromises viral clearance [57]. ZIKV structural proteins (C, prM and E) are the major targets of CD8+ T cell and CD4+ T cell responses [79].

ZIKV also elicits *humoral immune responses* by producing protective and neutralizing antibodies in humans [30,39]. However, this antibody-mediated protection effect against ZIKV could be jeopardized in individuals who have been pre-exposed to other flaviviruses such as DENV, which is the closest sibling of ZIKV. Those pre-existing neutralizing antibodies against DENV presented in those individuals could, instead of neutralize ZIKV, actually augment ZIKV infection and lead to more severe diseases [44]. This ADE effect of prior flaviviral infections on ZIKV pathogenicity have been thoroughly reviewed elsewhere [80,81].

Besides ZIKV-mediated inflammatory and humoral responses, ZIKV also triggers a series of *host cellular innate immune responses*, which are crucial for the recognition of viral invasion, activation of antiviral responses and determination of the final destination of viral infected cells (**Figure 1**). Primed by the pathogen-associated molecular pattern (PAMP) of different viruses, host cells recognize the invading virus by waking up different type of pattern recognition receptors (PRRs), which could be cell surface receptors or endosomal receptors. For example, ZIKV is recognized by an endosomal toll-like receptor 3 (TLR3), which is a PRR that specifically recognizes dsRNA virus [50,56,57]. TLR3 belongs to a class of endosomal receptors that can be found in cells of first responders such as macrophages or Langerhans cells. TLR3 activation plays a key role in host cell innate immune responses to viral infection. Consistent with the innate immune responses to dsRNA virus, ZIKV-induced TLR3 activation promotes phosphorylation of interferon regulatory factor 3 (IRF3) by TBK1 kinase leading to induction of type 1 interferon (IFN) signaling pathways [57,82]. This initial activation further activates RIG-I like receptors

(RLRs) responses in cytoplasm that subsequently induces transcription of RIG-I, MDA5 and several Type I and III IFN-stimulated genes including OAS2, ISG15, and MX1 [56]. Activation of Type I IFN signaling pathway results in the production and secretion of IFN-β. The secreted IFN-β in turn binds to IFN-β receptor, which activates JAK1 and Tyk2 kinases that phosphorylates STAT1 and STAT2. Upon ZIKV infection, the bindings of phosphorylated STAT1 and STAT2 heterodimer with IRF9 promotes ISRE3-mediated transcription of antiviral interferon stimulated genes (ISGS) [57]. One of the ISGS proteins viperin (virus-inhibitory protein, endoplasmic reticulum-associated, IFN-inducible) shows strong antiviral activity against ZIKV. Specifically, it restricts ZIKV viral replication by targeting the NS3 protein for proteasomal degradation [83]. Therefore, the production of TLR3- and RIG-1/MDA5-mediated type I IFN production and subsequent activation of the JAK/STAT innate immune pathway confer increased resistance to ZIKV infection [84].

ZIKV is a membrane-associated virus that utilizes ER for its replication and reproduction along the cellular secretory pathway. Through those cellular membrane interactions, ZIKV triggers autophagy, a cellular process that is normally involved in removal of aggregated or erroneously folded proteins through lysosomal degradation. Activation of cellular autophagy is a hallmark of flavivirus infection, which was thought to be part of the host innate immune response to eliminate invading intracellular pathogens [32,85-87]. Because autophagy activation could halt cellular growth and trigger apoptosis, ZIKV-induced autophagy was implicated in the ZIKV-mediated microcephaly [53,86,87]. Activation of autophagy elicits antiviral activities by removing viral proteins through reticulophagy, a selective form of autophagy that leads to ER degradation or inclusion of viral proteins in autophagosomes where viral proteins are destined for lysosomal degradation [88]. The ER-localized reticulophagy receptor FAM134B serves as a host cell restriction factor to ZIKV and other flaviviruses [89]. However, ZIKV-induced autophagy could be a double edged sword, which shows activities of both pro- and anti-ZIKV infection [88]. Activation of cellular autophagy counteracts ZIKV infection by actively removing viral proteins. As part of the host cell antiviral responses, Type I IFN signaling also limit ZIKV replication by promoting autophagic destruction of the ZIKV protease (NS2B3) in a STAT1-dependent manner [90]. Conversely, ZIKV takes advantage of formation of autopahgosomes where viral replication takes place [56]. ZIKV activates autophagy through the cellular mTOR stress pathway that connects oxidative stress and the ROS production. This virus-host interaction appears to be highly conserved because, in human fetal neural stem cells, ZIKV triggers autophagy through inhibition of the mammalian mTOR pathway *via* AKT [86]. In fission yeast, the ZIKV effect on TOR was also mediated through a parallel pathway *via* Tor1 and Tip41, which are the human equivalents of TSC1 and TIP41 proteins [91,92]. Altogether, ZIKV infection elicits RIG-1/MDA5- and TLR3-mediated innate immune responses leading to releases of Type I and Type III IFNs to protect cells from viral invasion. In the meantime, ZIKV triggers cellular activation of stress TOR signaling

pathway that induces autophagy. The balance between the pro- and anti-ZIKV activities of autophagy, at least in some cells, determines whether infected cells are protected through viral elimination or whether they are destined to apoptosis as the result of viral propagation in host cells.

4. Viral Counteraction to Host Antiviral Responses and ZIKV-induced Cytopathic Effects

Viral counteraction to host antiviral responses. To establish successful viral infection, the virus has adapted various strategies to counteract host antiviral responses (**Table 4**). The final infection outcome will depend on the balance between the host antiviral responses and the viral counteracting actions. A number of ZIKV-mediated counteracting actions are known. For example, once ZIKV infection is successfully established, it becomes resistant to IFN treatment suggesting ZIKV might have deployed effective counteractive measures against host innate immune responses [77,93]. As results, no secreted Type I and Type III IFNs were detectable from ZIKV-infected cells [57]. Indeed, ZIKV impairs the induction of Type-I IFN by binding to IRF3, a member of the interferon regulatory transcription factor (IRF) family [43,93,94]. These ZIKV-mediated counteracting effects are achieved through multiple ZIKV proteins (NS1, NS2A, NS2B, NS4A, NS4B, and NS5). All of these ZIKV proteins suppress, to various degrees, the IFN-β production by targeting distinct components of the RIG-I pathway [43]. For instance, the NS1, NS4A and NS5 proteins specifically inhibit IRF3 and NFkB [93], and the NS1 and NS4B proteins block the IRF3 activation [43,90]. Interestingly, a NS1 mutation A188V, which was found during the ZIKV epidemic starting in 2012, showed enhanced activity to block inhibits IFN-β induction and facilitated mosquito-mediated virus transmission [43]. This acquired mutation enables NS1 binding to TBK1 and reduces TBK1 phosphorylation. Reversion of this mutation back to the pre-epidemic genotype weakens the ability of ZIKV to counteract IFN-β production. Consistent with the notion that ZIKV blocks the IFN-β production through IRF3, the IRF3 knockout cells lost this ZIKV effect [42,43].

ZIKV also develops ways to block the JAK/STAT pathway [57] (**Figure 1**). For example, it blocks JAK1/Tyk2-mediated STAT1 and STAT2 phosphorylation that results in shutdown of ISGF3 transcription and translation of ISGS [57]. On one hand, ZIKV utilizes its protease to inhibit JAK1 kinase [90]. On the other hand, ZIKV uses NS5 protein to target STAT2 through direct binding and to promote its proteasome-mediated degradation [93-95].

ZIKV-induced cytopathic effects. Persistent viral replication and propagation inevitably confer adverse cytopathic effects to host cells. ZIKV, like many other viruses, encodes a limited number of proteins. To ensure successful viral replication, conceivably, it has to rely on host cellular resources to complete its

life cycle. Thus, it takes a variety of devious approaches to create a cellular environment for the benefit of its own reproduction. One common viral strategy is to deter host cellular growth, or to subvert host cell cycle into a specific phase of the cell cycle where the virus gains optimal benefit by maximizing availability of the cellular resources for its transcription, translation and assembly. This is indeed true for ZIKV that ZIKV infection to host cells such as NPCs restricts host cellular growth, induces cell cycle dysfunction and apoptosis [36,54,55]. Further, these ZIKV-mediated CPEs appears to associate with clinical manifestation of neurologic disorders such as microcephaly [36,96]. For instance, ZIKV-induced CPEs correlate with the decrease of neuronal cell-layer volume of the brain organoids resembling microcephaly, supporting ZIKV-induced microcephaly is likely the result of ZIKV-mediated increase of CPEs [37,49,50,53].

Although ZIKV confers various CPEs as described above, which ZIKV protein(s) is responsible for those specific CPEs, and how those ZIKV-mediated effects take place through interactions with host cells are elusive. To assist in identifying which ZIKV viral protein(s) is responsible for those observed CPEs, we embarked on a rapid genome-wide analysis of ZIKV proteins by using fission yeast (*Schizosaccharomyces pombe*) as a surrogate system [6,97]. Fission yeast is particularly useful here because those ZIKV-mediated CPEs affect highly conserved cellular activities among all eukaryotes [98-100]. Each one of the 14 ZIKV viral cDNA that encodes a specific ORF or small peptide was cloned into the fission yeast gene expression systems [101,102]. All of the ZIKV viral activities were measured concurrently under the same inducible conditions thus it allows functional characterization of the ZIKV proteins simultaneously. Consistent with the notion that ZIKV is a cellular membrane-associated virus and ER is the major “viral factory” [32,85,103], 9 out of the 14 ZIKV proteins and peptides associate with the ER network, including the nuclear membrane, ER to Golgi[32,103,104]. Seven ZIKV proteins including five mature and immature structural proteins (anaC/C, prM/M and E) and two non-structural proteins (NS2B and NS4A) conferred some of those same CPEs as reported in the ZIKV-infected mammalian cells [6,32,36,37,49]. Specifically, they also restricted cellular growth, triggered cellular autophagy, caused cell hypertrophy, and induced cell cycle dysfunction and cell death [6]. As described below, some of the same ZIKV protein-mediated CPEs have also been reported in mammalian cells.

The structural proteins. The yeast study showed (**Table 4**) that both the anaC and C proteins localize in the nuclei and trigger cellular oxidative stress leading to cell death [6]. Consistently, C is present in the nucleoli of human NPCs, a sub-nuclear structure where ribosome biogenesis takes place and plays a role in cellular response to stress [105]. The presence of C in nucleoli was associated with

activation of ribosomal stress and apoptosis [105]. Deleting part of the C protein prevented nucleolar localization, ribosomal stress and apoptosis [105].

The E protein is a major viral surface protein that is responsible for the viral entry. Thus it is a crucial viral determinant for initiating the ZIKV-host interaction. Sequence and structural comparisons of the E protein with that of other flaviviruses suggest it is overall unique among flaviviruses, although parts of it resemble its homologues of WNV, JEV or DENV [106,107]. During flaviviral assembly, E interacts with prM to form the prM-E heterodimers that protrude from the viral surface in the non-infectious and immature viral particles [11]. It is also involved in fusing the viral membrane with the host endosome membrane. Like other flaviviruses, the E protein is glycosylated at amino acid N154. The E glycosylation appears to be critical for ZIKV infection of mammalian and mosquito cells, because a glycosylation mutant N154Q diminished oral infectivity by *A. aegypti* vector and showed reduced viremia and diminished mortality in mouse models [108]. Interestingly, knockout of E glycosylation does not significantly affect neurovirulence in mouse models [108]. Instead, ZIKV with a non-glycosylated E protein is attenuated and defective in neuro-invasion because the mutant viruses replicate poorly in the brain of infected mice when inoculated subcutaneously. Conversely, it replicated well following intracranial inoculation, suggesting possible involvement of E in passing through the blood-brain barrier [109]. Furthermore, ZIKV viral particles lacking the E protein glycan were still able to infect Raji cells expressing the lectin DC-SIGN receptor, indicating the prM glycan of partially mature particles can facilitate the viral entry [110]. The E protein, in particular its extended CD-loop, may associate with the viral stability, cell cycle-dependent viral replication and *in vivo* pathogenesis, as disruption of these two regions by mutagenesis showed that shortening the CD-loop destabilizes the virus, and Δ346 mutant in this loop disrupts thermal stability of the virus [111].

In DENV, the prM protein forms a heterodimer with the E protein and affects viral particle formation and secretion [11]. The resultant non-infectious and immature viral particles are transported through the TGN, where prM is cleaved by a host protease Furin, resulting in mature infectious particles [12,13]. The transition from prM to M *via* the cleavage of host protease Furin is required for viral infectivity [8,10]. Therefore, both prM and M play important roles in viral pathogenesis. Consistent with the prM/M activities in host cells, in the yeast study, we showed that both prM and M proteins localize in ER [6]. Similarly, prM also localizes in ER in Vero cells [41]. In addition, the prM protein restricts cellular growth, affects cell cycling leading to cell death in the yeast [6]. So far, no description has yet been reported on the effect of individual prM or M protein on those basic cellular functions in mammalian cells. However, mutational analysis shows that the activity of prM protein contributes to fetal microcephaly [90]. Specifically, evolutionary analysis shows that a S139N substitution in the prM protein has persisted in the circulating ZIKV strains since the 2013 outbreak in French Polynesia to the subsequent spread to the Americas. A

single serine(S)-to-asparagine(N) substitution (S139N) in the viral polyprotein of a presumably less neurovirulent and Cambodian $ZIKV_{FSS13025}$ strain [112], substantially increased ZIKV infectivity in both human and mouse NPCs and led to more severe microcephaly in the mouse fetus, as well as higher mortality rates in neonatal mice [90]. Results of this study underscore the important contribution of prM to fetal microcephaly. However, how exactly the function of prM protein contributes to microcephaly and what is the functional impact of S139N mutation on the prM functions are presently unclear. It is intriguing to note that the residue of 139 is actually located in the Pr region of the prM protein. Since neither prM nor Pr is present in the mature and infectious viral particles [12,13], it would be interesting to learn the molecular mechanism underlying the effect of the S139N mutation on the increased viral infectivity.

The non-structural proteins. The ZIKV PR, which consists of a 40 residues of the NS2B cofactor and the NS3Pro domain of the NS3 [113], has been actively investigated for its PR activities (**Table 4**) [114-116]. Besides ZIKV PR-mediated viral proteolysis for viral reproduction, ZIKV PR also cleaves the ER-localized reticulophagy receptor FAM134B to counteract host cell restriction through a selective form of autophagy known as reticulophagy [89]. Indeed, depletion of FAM134B by RNAi significantly enhanced ZIKV replication [89]. The production of the same PRs produced by other flaviviruses causes cell death by apoptosis [117,118]. However, whether ZIKV PR causes apoptosis is presently unknown. The yeast study showed that expression of the *NS2B* gene, which encodes the co-factor of the ZIKV PR, does induces cellular autophagy and cell death [6]. It would be of interest to test whether fully active ZIKV PR can induce cell death in yeast and mammalian cells.

The NS4A, in conjunction with NS4B, activates cellular autophagy through inhibition of the mammalian TOR pathway *via* AKT [86]. Similarly, NS4A also inhibits the Tor1 pathway in the fission yeast. Furthermore, the yeast study showed that the inhibitory NS4A effect on TOR was mediated through Tor1 and Tip41, which are the human equivalents of TSC1 and TIP41 proteins [91,92].

Expression of NS2A reduces the cell proliferation and causes premature differentiation of radial glial cells in the developing mouse brain [90]. In addition, NS2A interacts with adherens junction (AJ) proteins that are present at the epithelial-endothelial cell junctions. This interaction results in degradation and malformation of the AJ complex [90]. This NS2A-induced growth deficiencies in the embryonic mouse cortex is unique to ZIKV, as the same effects were not found in the DENV. These NS2A effects could be part of the pathogenic mechanism underlying ZIKV infection in the developing mammalian brain [90].

The NS1 is a highly conserved protein among flaviviruses. It is an essential viral glycoprotein that plays a major role in virus-host interaction as it participates in viral replication, pathogenesis and immune evasion [119]. Like other flaviviruses, NS1 is expressed on the cell surface and exists in diverse forms. Intracellular NS1 exists as a dimer that is required for viral replication, whereas the secreted NS1 hexamer interacts with host factors and plays a role in immune evasion [119,120]. Freire *et al*. [121] first

unrevealed adaptation of the NS1 codon usage to human housekeeping genes in ZIKV Asian lineage, which could facilitate viral replication in humans. Indeed, an alanine(A)-to-valine(V) amino acid substitution at residue 188 (A188V) of the NS1 protein was acquired by the ancient ZIKV strain since the turning of the century in Southeastern Asia. This A188V-carrying ZIKV strain was circulated in that region before being disseminated to Southern Pacific islands and the Americas [122]. The residue 188 is located within the interface between two NS1 monomers. However, this A188V substitution does not affect NS1 dimerization but instead increase its secretability [42]. Strikingly, those A188V-carrying ZIKV epidemic strains were much more infectious in mosquitoes (*A. aegypti*) than the earlier Cambodia $ZIKV_{FSS13025}$ strain, resulting in increased NS1 antigenemia. Enhancement of NS1 antigenaemia in infected hosts promotes ZIKV infectivity and prevalence in mosquitoes, which could have facilitated transmission during the recent ZIKV epidemics [42]. Consistent with this notion, acquisition of the A188V substitution also correlates with enhanced ZIKV evasion of host interferon induction [43].

Also in line with the idea that the NS1 mutations might contribute to the enhanced viral pathogenicity leading to fetal microcephaly, another pathogenic mutation T233A was isolated from the brain tissue of ZIKV infected fetus with neonatal microcephaly [41]. The T233A mutation, also located at the dimer interface, is a unique residue for ZIKV but it was not found in other flaviviruses. The finding of this unique mutation could potentially be significant because T233 organizes a central hydrogen bonding network at NS1 dimer interface. The T233A mutation disrupts this network and destabilizes the NS1 dimeric assembly *in vitro* [41]. However, its actual pathogenic potential has not yet been tested. Together, these studies on the NS1 protein support the idea that ZIKV has acquired specific mutation(s) that increases its ability to evade host immune response and favors persistent infection leading to enhanced viral pathogenicity.

5. Concluding Remarks

Since the global ZIKV pandemic in 2015, an unprecedented and world-wide effect is being made to understand the etiology of this harmful virus. We have learned a great deal about its epidemiology, genomic sequences, clinical manifestation and pathogenicity. In this article, we describe molecular interactions of ZIKV with its host cells. In particular, we briefly outline different cell types and receptors that ZIKV utilizes for viral entry and infection. We then describe host cellular and immune responses to fight against ZIKV invasion. In contrast, ZIKV has adapted various counteracting strategies to defeat those host antiviral responses. Tilt of the final balance between the host antiviral defense and viral offensive retaliation will determines the outcome of viral survival and fate of the infecting cells. The level

of persistent viral replication and propagation inevitably cause various damages host cells, tissues and organs that ultimately lead to various diseases we described here including fetal microcephaly and other neurologic disorders. Yet, we are only scratching the surface of the molecular mechanism underlying its interactions with host cells. Much work still remains to answer some of those same questions as we asked at the beginning, *e.g.*, 1) what specific virologic changes have taken place that transformed the ZIKV from a begin virus to a highly pathogenic virus? 2) how a single viral mutation or combination of those acquired viral mutations as described in this review could change the viral pathogenicity leading to those recently discovered neurologic disorders? and 3) what specific changes during the ZIKV-host interactions finally tilted its balance and resulted in the enhanced CPEs and viral pathogenicity? There is little doubt that ongoing and future research will continue to provide answers to these questions. We hope this review could be a helpful reference to those who study ZIKV-host cell interactions, and the information described here could help to stimulate additional studies focusing on the molecular mechanisms of this virus.

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We want to thank all of those who have contributed our understanding of the emerging Zika virus especially those studies molecular mechanism underlying Zika virus-host interactions. We also want to apologize in advance if we have missed any important references that we should have included in this review. This work was supported in part by funding from the National Institute of Health (NIH R21 AI129369) and University of Maryland Medical Center (to R.Y.Z). S.B. was an awardee of the French Erasmus Programmes, Processus Infectieux en Milieu Insulaire Tropical (PIMIT), Université de La Réunion –INSERM, LA REUNION, FRANCE.

Conflicts of Interest

The authors declare that there is no conflict of interest of any kind. The opinions expressed by the authors contributing to this journal do not necessarily reflect the opinions of the institutions with which the authors are affiliated.

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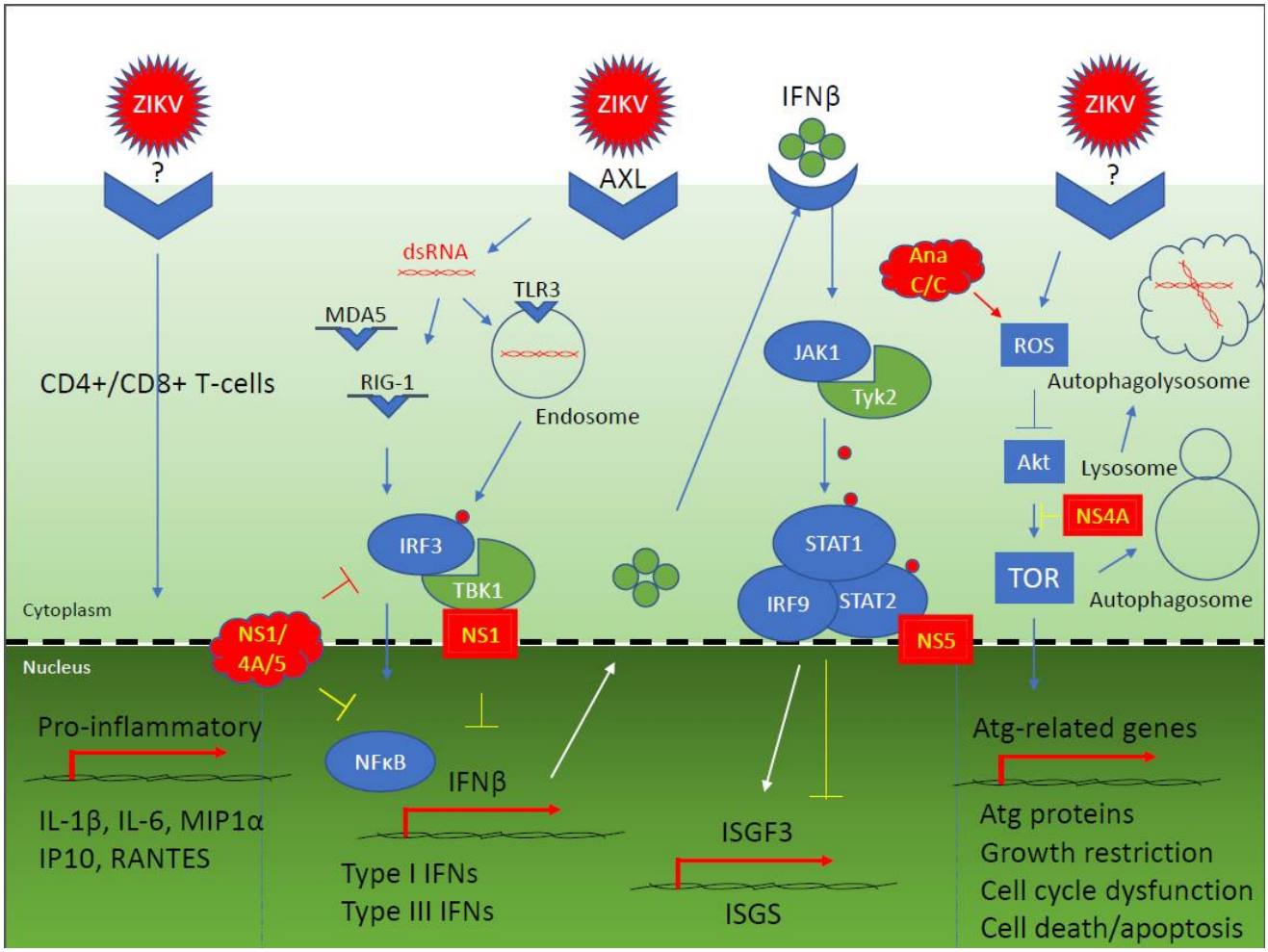
544 **Figure legend**



545

546 **Figure 1.**

547 This figure illustrates Zika virus interactions with host cells. The Zika virus or proteins are colored in red.
548 Cellular receptors or proteins that are affected by ZIKV are shown in blue. Cellular proteins shown in
549 green are regulators such as kinases. Three Zika viruses are used here to show ZIKV-induced T-cell
550 responses (left), ZIKV-mediated Type I and Type III IFNs productions (middle) and ZIKV-triggered
551 autophagy (right). → indicates a positive interaction. ┤ denotes inhibitory action. Small red dots are used
552 to indicate phosphorylation.

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554 **Table 1.** Zika viral strains that are known to cause microcephaly or microcephaly-like phenotypes

ZIKV Strain	Model used	Host/Location/Year	Microcephaly-like phenotypes	Reference
Human fetal tissue or organoid models				
MR766	Human brain-specific organoids	Rhesus monkey/Uganda/1947	Increased cell death and reduced proliferation, resulting in decreased neuronal cell-layer volume resembling microcephaly.	[49]
MR766	Human neurospheres and organoids	Rhesus monkey/Uganda/1947	Growth impairment of neurospheres and organoids	[37]
MR766	Human cerebral organoids	Rhesus monkey/Uganda/1947	Reduction of organoid growth and volume reminiscent of microcephaly via induction of TLR3	[50]
FSS 13025	Human brain-specific organoids	Human/Campodia/2010	Increased cell death and reduced proliferation, resulting in decreased neuronal cell-layer volume resembling microcephaly.	[49]
ZIKV(BR)	Human organoids	Human/Brazil/2015	Reduction of proliferative zones and disrupted cortical layers; induction of apoptosis, autophagy and impaired neurodevelopment	[53]
KU527068	Aborted human fetal brain	Human/Brazil/2016	Microcephaly with calcification in the fetal brain and placenta	[28]
FB_GWUH	Aborted human fetal brain	Human/USA/2016	Fetal brain abnormalities with diffuse cerebral cortical thinning	[123]
Mouse models				
PF/2013/KD507	Mouse	Human/French Polynesia/2013	Fetal demise or intrauterine growth restriction	[29]
ZIKV(BR)	Mouse	Human/Brazil/2015	Intrauterine growth restriction, including signs of microcephaly and vertical transmission	[53]
SZ01	Mouse vertical transmission	Human/Samoa/2016	Infection of radial glia cells of dorsal ventricular zone of the fetuses resulting in reduced cavity of lateral ventricles and decreased cortical surface area	[49]
SZ01	Embryonic mouse brain	Human/Samoa/2016	Cell cycle arrest, apoptosis, and inhibition of NPC differentiation, resulting in cortical thinning and microcephaly	[54]
CAM/2010 And VEN/2016	Neonatal mouse brain	Human/Venezuela/2016	Neonatal ZIKV infection of VEN/2016 leads to more severe microcephaly than CAM/2010. VEN/2016 strain infection leads to stronger immune response, more severe calcification, more neuronal death and abolished oligodendrocyte development, but less activation of microglial cells.	[124]

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555 **Table 2.** Cellular targets of Zika virus and receptor usages

Primary Cells	Receptor		Reference
Brain			
Neural progenitor cells (NPCs)	AXL, TLR3		[70,74,75]
Astroglial cells	AXL		[32,71,78,125,126]
Microglial cells	AXL		[71]
Placenta			
Hofbauer cells	AXL,Tyro3, TIM1		[59,60,73]
Trophoblasts	AXL,Tyro3, TIM1, TLR3, TLR8		[59,60,73]
Endothelial cells	AXL,Tyro3, TIM1		[29,73]
Skin			
Dermal fibroblasts	AXL, TIM-1, TYRO3, TLR3, RIG-I, MDA5		[56,127]
Epidermal keratinocytes	AXL, TIM-1, TYRO3, TLR3, RIG-I, MDA5		[56]
Immune system			
Immature dendritic cells	DC-SIGN		[56,57]
Dendritic cells	DC-SIGN		[58]
CD14+ monocytes	Unknown		[128-130]
CD14+CD16+ monocytes	Unknown		[129]
Testis			
Sertoli cell	AXL		[24,131,132]
Spermatozoa	Tyro3		[133,134]
Kidney			
Renal mesangial cell	Unknown		[135]
Glomerular podocytes	Unknown		
Renal Glomerular Endothelial Cell	Unknown		
Retina			
Retinal pericytes	Tyro3, AXL		[1,136]
Retinal microvascular endothelial cells	Tyro3, AXL		
Permissive human cell lines			
Cell line	**Origins**	**Permissiveness**	**Reference**
SK-N-SH	Brain / Bone marrow	**	[137]
SH-SY5Y	Nerve	**	[138]
SF268	CNS in brain	***	[36,62]
HBMEC	Brain	***	[61,132]; our unpublished data
SNB19	CNS in brain	***	[36]
Huh-7	Liver	***	[62]
HFF-1	Skin	***	[56]
A549	Lung	***	[77,138]
HOBIT	Osteoblast-like Cells	***	[139]

Note: **, moderate permissive; ***, highly permissive

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558 **Table 3.** Host cellular responses to Zika viral infection

Response	Proteins involved	Molecular actions and consequences	Reference
Pattern recognition receptors (PRRs)			
TLR3-mediated response	IRF3, TBK1	Early response that triggers IRF3, recognize dsRNA viruses and PAMPs; induce type I interferons (INF-beta); phosphorylation of STAT1 and STAT2	[50,56,57]
RIG-I like receptors (RLRs), RIG-I and MDA5	IRF-3, NF-kB, IFN-beta	Late response; also recognize invasion of dsRNA viruses	[56,57]
Type I and Type III interferon activation	OAS2, ISG15, MX1	Production of IFNβ and activation of antiviral responses	[56]
Host Antiviral Cellular and Immune Responses			
Pro-inflammatory responses	Cytokines: IL-1β, IL-6, MIP1α; chemokines: IP-10, RANTES	ZIKV induces pro-inflammatory responses in a cell type-specific manner	[57,76]
Neutralizing and protective humoral immune responses	IgM, IgG	Cross reactivation of antibodies to ZIKV, DENV and WNV; Possible support of the "Antibody-dependent enhancement (ADE)" hypothesis	[140-142]
CD8+ T-cell immune responses	Cytokines (IL-2, IL-4, IL-9 and IL-17) that promote polyfunctional T-cell responses	Activation of polyfunctional T-cell antiviral responses	[76,143,144]
CD14 + monocytes prime NK cell activity	CXCL9, CXCL10, CXCL11, CCL5, IL-15	NK cells are activated in ZIKV-infected patients. ZIKV-infected macrophages are primed to “communicate” with NK cells.	[130]
Counteraction by ZIKV			
Anti-Type 1 IFN signaling	anti-JAK/STAT signaling (Bowen, 2017)/anti-IRF3 and NF-kB signaling	See Figure 1 for details	[93,145,146]
Inhibit interferon-β production	ZIKV NS5 (all type)/IRF3 and NS1 from ZIKV strain since 2012/TBK1	1) ZIKV NS5 suppresses IFN-β induction through binding to IRF3. 2) Mutation position 188 modulates NS1 to subvert IFN-β activation. This mutation (188-Val, since 2012) enables NS1 binding to TBK1 and reduces TBK1 phosphorylation = antagonizes IFN-β production	[43]
Anti-Type III IFN signaling	IFNs	See Figure 1 for details	[145]
Autophagy	mTOR, AKT, TSC1, TIP41	Autophagosome formation; increase of viral replication; Torin 1, an autophagy inducer vs 3-methyladenine inhibitor	[6,86]

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564 **Table 4.** ZIKV proteins and associated cytopathic effects

Protein	Primary function	Main cytopathic phenotypes	Reference
Structural Proteins			
AnaC	Precursor structural Protein	A putative cytopathic factor based on a yeast study	[6]
C	Mature structural Protein	A putative cytopathic factor based on a yeast study; Induce ribosomal stress and apoptosis	[6,105]
PrM	Precursor structural Protein	A putative cytopathic factor based on a yeast study; a single prM mutation contribute to fetal microcephaly	[6,90]
M	Mature structural Protein	A putative cytopathic factor based on a yeast study	[6]
Pr	Cleaved product	Unknown	
E	Mature structural Protein	A putative cytopathic factor based on a yeast study A single residue in the αB helix of the E protein is critical for Zika virus thermostability; interaction with host cell membrane	[6,147]
Non-structural Proteins			
NS1	Virus-host interaction as it participates in viral replication, pathogenesis and immune evasion	An essential role in viral replication and immune evasion. A188V substitution might be responsible for enhanced ZIKV infectivity and enhanced evasion of host IFNβ production; A Zika Vaccine Targeting NS1 Protein Protects Immunocompetent Adult Mice in a Lethal Challenge Model; a T233A mutation destabilizes NS1 assembly in neonatal microcephaly	[41-43,148]
NS2A	Unknown	Zika-Virus-encoded NS2A disrupts mammalian cortical neurogenesis by degrading adherens junction (AJ) proteins, leading to reduced proliferation and premature differentiation of radial glial cells and aberrant positioning of newborn neurons.	[90]
NS2B	Protease	Form a protease complex with NS3; a putative cytopathic factor based on a yeast study	[6,115]
NS3	Helicase	Counteracting the JAK/STAT signaling by interacting with JAK1 for its degradation; conversely, type I INF signaling promotes the autophagic destruction of NS2B3 in a STAT1-dependent manner, thus limiting ZIKV replication.	[90]
NS4A	Viral RNA synthesis and viral morphogenesis	Induce autophagy and inhibit TOR pathway through Akt and/or PP2A activator Tip41	[6,86]
2K	A signal peptide for NS4B	Viral RNA synthesis and viral morphogenesis	[6,8,9]
NS4B	Viral RNA synthesis and viral morphogenesis	Synergistic to NS4A on TOR pathway	[86]
NS5	Methyltrasferase; RNA-dependent polymerase	Suppressor of type 1 and type III IFN signaling; activate type II IFN signaling; Binding to STAT2 for its degradation. Suppresses IFN-β induction through binding to IRF3	[94] [43]

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