

**Authors:** Karli Theberge,<sup>1</sup> Sarah Derby,<sup>1</sup> Emily Ramirez,<sup>1</sup> \*Meghan May<sup>1,2</sup>

1. College of Osteopathic Medicine, University of New England, Biddeford, Maine, USA  
2. Center for Excellence in Public Health, University of New England, Portland, Maine, USA  
\*Correspondence to [mmay3@une.edu](mailto:mmay3@une.edu)

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## Abstract

Zika virus (ZIKV) is a flavivirus that was met with relatively little acclaim when it was discovered in 1947. Initial clinical reports of ZIKV included asymptomatic infection or mild, febrile illness; however, the view of ZIKV as an insignificant virus changed dramatically following the epidemic in the Western Hemisphere that started in 2015. This epidemic featured central nervous system involvement in children and adults, and a devastating congenital syndrome following infection of pregnant women. While the pathogenicity of ZIKV was virtually undescribed prior to this epidemic, in the past few years, numerous reports have described receptor-ligand interactions, aspects of tissue tropism, host-pathogen interactions, and diversity across viral clades. In this paper, the variety of clinical presentations and virulence determinants of ZIKV are reviewed.

## HISTORY AND EPIDEMIOLOGY

Zika virus (ZIKV) was first isolated in 1947 from rhesus monkeys during a routine surveillance of yellow fever in the Zika Forest of Uganda. A year later, ZIKV was recovered from the mosquito species *Aedes africanus* in the same area.<sup>1,2</sup> By 1952, the first human cases had been detected in the United Republic of Tanzania, Uganda, and India via antibody neutralisation tests. Throughout the 1950s, human serology detection of ZIKV in adults and children arose in Nigeria, the British colonies of Malaya and North Borneo (both currently part of Malaysia), the Philippines,

Nigeria, Egypt, Vietnam, and Mozambique.<sup>3</sup> In 1958, two additional strains were isolated from *A. africanus* species that were collected in the Zika Forest.<sup>4</sup> The first report of human illness caused by ZIKV came in 1964 and consisted of a mild febrile state and maculopapular rash, thereby confirming that ZIKV is a causative agent of human disease.<sup>1,3,5</sup>

From 1960 through to the early 2000s, ZIKV was sporadically detected in humans via haemagglutination inhibition and other serological methods.<sup>3,6</sup> Symptomatic cases were rare, leading to the relatively benign designation of ZIKV disease. ZIKV was continuously isolated

from sentinel rhesus monkeys that were used for field research, as well as from numerous mosquito species, predominantly of the genus *Aedes*, in several African countries. By the end of the 20<sup>th</sup> century, the geographical distribution of ZIKV expanded throughout equatorial Asia, with confirmed widespread population exposure in Indonesia, Malaysia, and Pakistan. This included ZIKV detection in mosquitoes and sporadic human cases, but no epidemic disease.<sup>3</sup>

The first widespread epidemic associated with ZIKV occurred on the Pacific island of Yap in 2007.<sup>7,8</sup> During this time, 185 suspected cases of ZIKV infections were recorded, 49 of which were confirmed via PCR and 59 of which were presumed probable in patients with the IgM antibody against ZIKV; no deaths were recorded.<sup>3</sup> The introduction of ZIKV to the Yap population was suspected to be caused by travel and trade, either through infected humans or mosquitoes. This theory was reinforced by the lack of monkeys present on the island that could act as sentinel reservoirs, as well as the publication of two geographically distinct ZIKV lineages in 2012, each with multiple strains. Nucleotide sequencing of ZIKV isolates from multiple countries provided strong evidence that the ZIKV strains responsible for the epidemic in Yap emerged from Southeast Asia.<sup>3,9</sup>

ZIKV outbreaks in the Pacific islands continued to occur throughout the years that followed.<sup>3</sup> In March 2015, the largest and most recent ZIKV epidemic began in Brazil. The rapid spread of infection through *Aedes* mosquitoes and sexual transmission led to Brazil declaring a national public health emergency in November 2015. ZIKV then continued to rapidly spread throughout the Americas, prompting the World Health Organization (WHO) to declare a Public Health Emergency of International Concern in February 2016.<sup>10</sup> This ZIKV epidemic was associated with many new symptoms and lasting effects, provoking intense investigations. The reports that followed marked the associations of ZIKV with congenital syndromes, malformations, meningoencephalitis, and Guillain-Barré syndrome.<sup>3</sup> Retrospective analyses indicated that these syndromes were also present in the South Pacific in the preceding years, although strong associations were not made at the time.<sup>11</sup> In the spring of 2016, the European Union (EU) and the USA strongly advised that women in

any stage of pregnancy should postpone travel to countries with known local transmission of ZIKV. There is currently no vaccine or treatment for ZIKV, so it remains an ongoing concern and a potential threat to public health.<sup>10</sup>

## ZIKA VIRUS BIOLOGY, TRANSMISSION, AND CLINICAL PRESENTATIONS

ZIKV belongs to the genus *Flavivirus*, a taxon of single-stranded, enveloped RNA viruses that also includes dengue virus (DENV), yellow fever virus, West Nile virus (WNV), Powassan virus, and many others.<sup>12</sup> The transmission route of flaviviruses is primarily vector-borne, resulting in a wide range of clinical diseases.<sup>12,13</sup> ZIKV is primarily transmitted by *Aedes* species mosquitoes, specifically *Aedes aegypti* and *Aedes albopictus*.<sup>13,14</sup> Additional routes of flavivirus transmission include horizontally from mother to fetus, via blood or issued products containing the virus, and, for certain species such as ZIKV, sexual transmission.<sup>13</sup> Once inside the human host, flaviviruses are able to enter host cells through receptor-mediated endocytosis and utilise the cells' resources in order to replicate and promote further infection.<sup>15</sup>

Clinical disease produced by ZIKV and related flaviviruses varies from asymptomatic or mild symptoms to severe nervous system deficits and fetal deformities. Mild symptoms include fever, rash, headache, arthralgia, myalgia, conjunctivitis, and uveitis.<sup>14,16</sup> Flaviviruses are a neuroinvasive group, and infections can potentially culminate in severe neurological diseases, including encephalitis, meningitis, and seizures.<sup>16,17</sup> Pregnant women have a risk of transmitting the virus to the fetus, resulting in adverse pregnancy outcomes for some viruses and congenital Zika syndrome for cases of ZIKV. The effects of ZIKV on a developing fetus have been found to focus around the nervous, musculoskeletal, and visual body systems, though effects can be found throughout the body.<sup>18</sup> The most severe presentation of congenital Zika syndrome in liveborn infants is microcephaly.<sup>19</sup> Other notable neurologic manifestations include ventriculomegaly, hydrocephalus, and hypoplasia or atrophy of the cerebral cortex, cerebellum, and brainstem.<sup>18</sup> Congenital Zika syndrome has also presented with clubfoot, patent foramen ovale,

dysphagia, and hearing and vision loss.<sup>19</sup> The disease presentation of ZIKV infection correlates both with the predominant cell targets and the elicited immune responses of the host, making host cell binding and host-pathogen interactions critical factors.<sup>3,20</sup>

## ASPECTS OF ZIKA VIRUS VIRULENCE

### Host Cell Binding

The ZIKV genome encodes three structural proteins (capsid; precursor membrane [prM]; and envelope [E]), seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5), and two non-coding regions at the 3'- and 5'-terminal ends.<sup>21</sup> Host cell adsorption is mediated by the E protein. After infection, the E protein mediates the binding of ZIKV to host cell entry factors and surface receptors, before undergoing clathrin-dependent endocytosis.<sup>21</sup> A specific binding motif (E-N-R-A-K-V, E protein amino acid positions 162-167) both directly binds to ZIKV-permissive cell lines and significantly inhibits ZIKV adsorption *in vitro* by competing for the same receptor, strongly implicating this portion of the E protein as a functional driver of host cell binding. This motif is notably proximal to the asparagine at position 154, which is implicated in host cell interactions by the four DENV serotypes (DENV-1-4), and in the neuroinvasion of WNV and St. Louis encephalitis virus.<sup>22-24</sup> Flaviviruses use a variety of receptors to mediate entry into host cells, making it difficult to establish targets for the prevention of ZIKV infection.<sup>25</sup> ZIKV entry receptors include AXL, DC-SIGN, TYRO3, and TIM-2 among others. These receptors are present in cells of the brain, skin, testes, placenta, kidneys, retina, and immune system, with ZIKV favourably targeting primary human brain microvascular endothelial cells. The first described receptor was AXL; however, genetically ablated animals were still susceptible to infection, which indicated that multiple viral entry mechanisms must exist.<sup>21,25,26</sup>

Once infection is established in mammalian host cells, antiviral responses in the host are stimulated, leading to the activation of inflammation as well as humoral and innate immune responses. Inflammation is mediated by cytotoxic (CD8+) T cell activation, leading to downstream cytokine and chemokine release. The humoral immune

response includes the production of IgG and IgM protective antibodies against ZIKV, and the innate immune response is responsible for the recognition of ZIKV and activation of antiviral responses.<sup>25</sup> In order to bypass these host immune responses, ZIKV has established numerous mechanisms of evading destruction. For example, once ZIKV infection is established, it blocks Type I and Type III interferon (IFN) induction, which hampers the innate immune responses of the host and establishes resistance to IFN treatment. ZIKV also mediates the inhibition of the JAK-signal transducers and activators of transcription signalling pathway and IFN- $\beta$  production, and also induces cytopathic effects and apoptosis in human neural progenitor cells (hNPCs).<sup>25</sup> The infectious outcome of ZIKV is dependent on the balance between the antiviral immune responses of the host and the counteracting mechanisms of ZIKV.<sup>25</sup>

### Variations in Pathogenesis Across Zika Virus Lineages

There are two major phylogenetic lineages of ZIKV: the Asian and Western Hemisphere clade and the African clade. All ZIKV human epidemics to date have been caused by strains belonging to the Asian lineage, and only Asian clade strains have been linked to congenital abnormalities and neurological disorders.<sup>27</sup> The African clade strains have a higher transmissibility in *A. aegypti* mosquitoes; however, they also exhibit a lower replication efficacy in vertebrate cells compared to that of Asian strains, which may be a contributing factor in the reduced pathogenicity of the African strain in humans.<sup>27,28</sup>

The region of highest genetic variability amongst the African and Asian strains occurs in the 'pr' peptide portion of the prM protein.<sup>29</sup> The functional impact of this variability is widespread, and the evolutionary drivers are likely a combination of immune selection and changes in binding avidity and/or specificity. One position within the pr region with multiple known amino acid changes is position 17. The consensus sequence from African lineage strains show a serine at position 17, whereas Asian lineage strains have modified this to an asparagine. This change was strongly associated with increased neurovirulence and microcephaly in a fetal mouse model, likely by enhancing viral maturation kinetics during infection.<sup>30</sup>

Of the genetic differences that are conserved across strains of Asian and African lineages, the most extensively studied is the introduction of an N-glycosylation motif (sequence: N-X-S/T) at codons 154-156 of the E protein in the Asian lineage.<sup>28</sup> Changes in binding affinity or avidity and host cell tropism, most notably neuroinvasiveness, have been ascribed to the addition of an N-acetylglucosamine to the asparagine at position 154 in ZIKV and other neurotropic viruses.<sup>23,24,26</sup> In addition, the phosphatidylserine-binding protein annexin V was shown to competitively inhibit the infection of Vero cells by the Asian lineage strain PRVABC59, but did not affect the infection of the African lineage strain MR-766. This suggests that Asian lineage ZIKV can initiate host cell entry via phosphatidylserine-mediated adsorption. This is likely to occur via phosphatidylserine binding to the growth arrest-specific 6 (Gas6) protein, which in turn binds AXL, a tyrosine kinase receptor.<sup>26</sup> AXL downregulates the IFN signalling response, thereby accelerating infection. After binding to AXL, ZIKV enters the cell via clathrin-mediated endocytosis, and thereafter transfers to the Ras analog in brain 5-positive endosomes to establish infection.<sup>31</sup> This facilitates migration to the lymph nodes and then the bloodstream.<sup>11</sup> Compared with other flaviviruses, the Asian lineage ZIKV strain FSS13025 infects fetal endothelial cells more efficiently as it has a higher affinity to bind Gas6, which in turn aids in its interaction with AXL.<sup>32</sup> Taken together, the addition of an N-acetylglucosamine proximal to the E protein binding motif, the ability to enter host cells via phosphatidylserine-driven mechanisms, and the enhanced replication kinetics conferred by alterations in the prM protein plausibly explain the profound differences between the clinical presentations of the African and Asian lineage ZIKV strains.

### **Antibody-Dependent Enhancement**

Antibody-dependent enhancement (ADE) is the phenomenon of a virus stimulating non-neutralising antibodies in order to facilitate its entry into host cells by opsonisation, a process that involves the binding of antibodies to infectious viruses.<sup>33</sup> The non-neutralising antibodies required for this process do not necessarily have to derive from the virus or the strain utilising it, as long as the viral antigen

expresses cross-reactivity. ADE has been described in detail for other flaviviruses, most notably DENV-1-4.<sup>34</sup> Individuals infected with DENV generate antibodies during their primary infection, and non-neutralising antibodies facilitate a cell entry that is far more efficient upon reinfection. These enhanced replication dynamics lead to more severe disease, often seen clinically as a haemorrhagic fever as opposed to a simple viraemic fever.<sup>34</sup>

The occurrence of ADE during ZIKV infection is a subject of debate. ZIKV and DENV share numerous antigens, and antibodies from convalescent patients with DENV have been shown to bind ZIKV *in vitro*.<sup>35-40</sup> However, whether a previous DENV infection can lead to enhanced ZIKV disease still appears somewhat ambiguous. At least one study using a rhesus macaque model demonstrated enhanced disease at the maternal-fetal interface in monkeys exposed to DENV prior to infection with ZIKV,<sup>41</sup> and a lethal-challenge model showed elevated ZIKV titres and increased levels of proinflammatory cytokines in mice that received anti-ZIKV antibody infusions prior to the experimental infection.<sup>42</sup> Antibodies against WNV enhanced the cellular uptake of ZIKV, and hyperimmune sera from a large clinical trial of the attenuated DENV vaccine Dengvaxia® were similarly shown to enhance ZIKV entry and replication *in vitro*.<sup>43,44</sup> Despite these persuasive findings, a correlated increase in clinical severity during ZIKV infection post-DENV in humans remains unclear.<sup>45,46</sup> Multiple cases have been reported wherein antibodies from prior ZIKV infections led to enhanced, haemorrhagic disease upon infection with DENV, indicating that prior ZIKV infection can act analogously to prior DENV infection and lead to dengue haemorrhagic fever (DHF).<sup>47</sup> Animal models that have utilised mice and macaques as test subjects are highly consistent with these clinical reports.<sup>48-50</sup> Given the co-circulation of ZIKV and DENV in many parts of the world, the predisposition of convalescent patients with ZIKV to develop the higher-mortality DHF presentation upon infection with DENV is a matter of significant public health concern.

### **Pathologic Host-Pathogen Interactions**

The majority of non-congenital ZIKV infections are asymptomatic or associated with only mild symptoms. In contrast, fetal exposure to ZIKV

during the first trimester of pregnancy can present with central nervous system symptoms, including microcephaly and cortical malformations such as simplified gyral pattern, frontal lobe involvement, and ventriculomegaly.<sup>51</sup> The variety of clinical outcomes indicate a diversity in host-pathogen interactions across tissues, age ranges, and developmental stages. Skin fibroblasts, keratinocytes, and immature dendritic cells are all permissive to ZIKV infection and are the first cells encountered following inoculation via mosquito bite.<sup>52</sup> Host cell entry is mediated by multiple mechanisms as described above, but the application of AXL-mediated entry has notable implications both for inflammatory responses and for the fetus during infection. During mosquito-derived infection, AXL downregulates the IFN signalling response, thereby hampering early antiviral responses and facilitating infection. AXL also plays a role in the ability of ZIKV to cause the congenital disease that is distinctive among flaviviruses. ZIKV displays a higher affinity to bind the AXL ligand Gas6 than other flaviviruses, which gives it a greater capacity to infect placental and fetal endothelial cells.<sup>32</sup> Once the placental barrier has been breached by ZIKV, all host cell entry mechanisms can then be utilised to cause systemic fetal disease.

The mild, usually self-limiting symptoms of ZIKV infection indicate that the innate immune response plays a critical role in controlling ZIKV infections. One of the initial innate immune defences that has been implicated in the response to ZIKV infection is autophagy, which has been shown to increase during infection both *in vitro* and *in vivo*.<sup>53</sup> Melo et al.<sup>53</sup> reported that the vasodilatory peptides angiotensin-(1-7), which are downstream markers of induced autophagy, were increased in the serum of ZIKV-infected patients relative to healthy controls.<sup>53,54</sup> Due to the intricate, reciprocal regulation of inflammation and autophagy, it is tempting to speculate that the observed increase in autophagy is not necessarily unique to ZIKV, but rather a generic indicator of infection. However, stimulating autophagy by the inhibition of modified nucleoside transport resulted in a concomitant increase in ZIKV replication kinetics *in vitro*. This relationship persisted across eight cell lines in the absence of exogenous inflammatory signals, suggesting a strong functional association between autophagy and ZIKV replication.<sup>55</sup> This

association is also likely to be a contributing factor to congenital Zika syndrome, as several proteins critical to autophagy also mediate centrosome function.<sup>11</sup>

A major protective component of the innate immune response during ZIKV infection is the Type I IFN (IFN- $\alpha$  and IFN- $\beta$ ) system.<sup>56</sup> On activation, the induction of IFN regulatory factors and nuclear factor- $\kappa$ B occurs, which in turn induces other inflammatory cytokines and chemokines.<sup>57</sup> The ZIKV-induced expression of hundreds of IFN-stimulated genes (ISGs) affects the viral life cycle and viral replication due to their role in RNA processing. While the downregulation of IFN signalling early in infection has been observed *in vitro*, the ultimate expression of ISGs and mild clinical presentation indicate that infected individuals ultimately overcome the initial ZIKV-mediated inhibition. The activities encoded by ISGs in antigen-presenting cells (i.e., dendritic cells and macrophages) are important for T and B cell activation and the development of the adaptive immune response and subsequent virus clearance.<sup>57,58</sup> As dendritic cells and macrophages are preferred host cells for ZIKV during primary infection, the induction of Type I IFNs are critically important for both acute viral clearance and the generation of memory response. However, the stimulation of IFN expression leads to the upregulation of major histocompatibility complex (MHC) class I molecules during flavivirus infections, including ZIKV.<sup>59,60</sup> Increased MHC class I expression during ZIKV infection is followed by increased T cell lysis and the inhibition of natural killer (NK) cell activity.<sup>61,62</sup> Consistent with this, Glasner et al.<sup>60</sup> demonstrated that ZIKV infection went largely undetected by NK cells; therefore, by upregulating MHC class I molecules, ZIKV avoided early detection by NK cells and replicated quickly before T cell responses could be mounted.<sup>60</sup> Ultimately, however, the Type I IFN responses lead to the successful clearance of ZIKV in uncomplicated cases, largely due to the protective role of CD8+ cells.<sup>63,64</sup>

## TISSUE-SPECIFIC FINDINGS

### Nervous System

ZIKV has been associated with neuroinflammation in children and adults, resulting in meningitis, meningoencephalitis, and an increased number

of Guillain-Barré syndrome cases.<sup>11</sup> Fetal microcephaly cases also saw a marked increase due to maternal infection with ZIKV during the first trimester. Chimelli et al.<sup>65</sup> conducted an analysis of post-mortem infants with confirmed ZIKV infection during the first trimester, identifying ventriculomegaly due to damage to the midbrain with aqueduct stenosis or distortion, as well as small brains with ex-vacuo ventriculomegaly.<sup>65</sup> Well-formed brains with mild calcification were seen in infants where maternal infection occurred later. They also observed an absence of descending fibres consistent with spinal motor cell loss presenting as intrauterine akinesia, arthrogryposis, and neurogenic muscle atrophy. Altogether, these findings suggest that the central nervous system is vulnerable to ZIKV infection during early development.<sup>65</sup>

Absent or decreased Type I IFN responses early in infection amplify ZIKV replication, and central nervous system cells, specifically axons and myelinating oligodendrocytes, have an increased susceptibility when compared with peripheral nervous system cells.<sup>66</sup> ZIKV affects the central nervous system by directly infecting hNPCs that originate from pluripotent stem cells, causing hNPCs to release infectious ZIKV particles, and reducing hNPC numbers by decreasing cell growth, increasing cell death, and causing the dysregulation of cell cycle progression.<sup>67</sup> ZIKV affects haematopoietic cells with microglia, the innate macrophage population localised throughout the brain, and induces a proinflammatory state indicated by elevated immune mediators, such as IL-6, TNF- $\alpha$ , IL-1 $\beta$ , and monocyte chemoattractant protein 1.<sup>68</sup> In targeting central nervous system cells, especially human brain cells, ZIKV reduces their viability and growth, thereby reversing neurogenesis during human brain development.<sup>69,70</sup>

## Testes

Detection of viable ZIKV in semen, the demonstration of sexual transmission, and clinical reports of haemospermia all clearly indicate that ZIKV expresses gonadal tropism.<sup>11,71,72</sup> Convalescent patients exhibited lower sperm counts and increased sperm abnormalities that persisted for at least 3 months.<sup>73</sup> Insights into testicular pathophysiology and the mechanisms of infertility have been gained from animal models. A 2016 study by Ma et al.<sup>74</sup> that examined

ZIKV infection and male infertility in a murine model demonstrated that ZIKV infection can result in the production of pro-inflammatory cytokines and chemokines. These effects were most notable in the testes and epididymis, but not in the prostate or seminal vesicles. The study specifically identified that stem-like testicular peritubular myoid cells and spermatogonia are particularly vulnerable to ZIKV infection, and, in some cases, this can lead to infertility.<sup>74</sup> Another study indicated that ZIKV primarily infected spermatogonia, primary spermatocytes, and Sertoli cells, which caused the destruction of the seminiferous tubules and led to cell death.<sup>75</sup>

## Ocular Tissue

ZIKV can involve the eye during mosquito-transmitted or vertically acquired infections. In infected children and adults, ZIKV can cause primary conjunctivitis and uveitis, which are usually self-limiting.<sup>76</sup> In contrast, permanent ocular abnormalities have been detected in many confirmed cases of congenital Zika syndrome. The most common isolated and combined fetal fundus presentations included macular chorioretinal atrophy, chorioretinal atrophy elsewhere, focal pigmentary changes in the macular region, and optic nerve abnormalities.<sup>77</sup> A small 2016 report described three infants with congenital Zika syndrome who had unilateral ocular abnormalities indicating gross macular pigment mottling and foveal reflex loss.<sup>78</sup> A larger study in 2016 identified normal anterior segments in the infants examined; however, there were further occurrences of macular pigment mottling and/or chorioretinal atrophy, as well as optic nerve abnormalities such as optic disc hypoplasia, pallor, and/or an increased cup-to-disc ratio. One infant also presented with horizontal nystagmus.<sup>79</sup> Another 2016 study with a different cohort of patients showed similar findings; however one infant had bilateral iris coloboma and lens subluxation in one eye, indicating an anterior segment finding.<sup>80</sup>

## Placenta

Placental damage due to ZIKV infection is likely multifaceted. ZIKV entry via AXL and Gas6 and subsequent lytic replication can cause the necrotic cell death of both trophoblasts and fetal endothelial cells, ultimately compromising the integrity of the placenta.<sup>32</sup> In addition, ZIKV

infection of the placenta drives altered lipid metabolism pathways. The placenta has a high lipid content, and the metabolism of lipids supports fetal development. Disruption of the placental lipid metabolism has been shown to play a role in spontaneous pregnancy loss, intrauterine growth restriction, and other adverse pregnancy outcomes.<sup>81,82</sup> ZIKV infection during pregnancy leads to the reprogramming of the placental lipidome to a profile favourable to viral replication, mitochondrial dysfunction, and a dysregulated inflammatory response.<sup>83</sup>

## CONCLUSION

Though ZIKV was not a new virus when the epidemic emerged in the Americas in 2015, it clearly presented with an emerging neurologic and teratogenic pathology. Retrospectively, it is clear that the Asian lineage strains acquired capacities to more efficiently cross the blood-brain, blood-testis, and placental barriers relative to the ancestral African lineage. In this way,

ZIKV has gone from a virus causing a benign, self-limiting illness to a virus capable of causing lethal disease in adults, adverse pregnancy outcomes, and/or a severe congenital syndrome. The opinion of the authors is that alterations in tissue tropism and infectivity associated with novel binding partners or altered binding affinity likely led to these new clinical manifestations. Pathologic host responses following the manipulation of the immune response by ZIKV have also been described, and include the modulation of IFN responses and autophagy. Additionally, there is mounting evidence that previous exposure to ZIKV creates the potential for DHF in patients on exposure to DENV. The previously undescribed clinical presentations associated with Asian lineage ZIKV strains illustrate the potential for novel epidemic disease events that are associated with known viruses, and underscore the importance of understanding the pathophysiology of these infections.

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