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REVIEW

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Research advancements in the neurological presentation of flaviviruses

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Summary

Owing to the large-scale epidemic of Zika virus disease and its association with microcephaly, properties that allow flaviviruses to cause nervous system diseases are an important area of investigation. At present, although potential pathogenic mechanisms of flaviviruses in the nervous system have been examined, they have not been completely elucidated. In this paper, we review the possible mechanisms of blood-brain barrier penetration, the pathological effects on neurons, and the association between virus mutations and neurotoxicity. A hypothesis on neurotoxicity caused by the Zika virus is presented. Clarifying the mechanisms of virulence of flaviviruses will be helpful in finding better antiviral drugs and optimizing the treatment of symptoms.

KEYWORDS

flavivirus, mechanism, neurological presentation

1 | A BRIEF INTRODUCTION TO FLAVIVIRIDAE AND NERVOUS SYSTEM DISEASES

The name "flavivirus" is derived from the Latin word "flavus," meaning yellow, because of the jaundice caused by yellow fever virus (YFV). Flaviviruses are primarily transmitted by arthropods. Symptoms of flavivirus infection can range from mild fever and malaise to fatal encephalitis and haemorrhagic fever.[1]

Viruses in the genus *Flavivirus*, which contains more than 70 viruses, including the Japanese encephalitis virus (JEV), YFV, West Nile virus (WNV), dengue virus (DENV), and Zika virus (ZIKV) (Table 1), pose a serious threat to human health.[2-7] Of these viruses, ZIKV can be transmitted through sexual intercourse[8-10] and blood donation,[11] increasing the concern about possible outbreaks of ZIKV disease. By contrast with other flaviviruses, a neurological presentation of disease occurs more often with ZIKV disease. In addition to neurological symptoms such as Guillain-Barré syndrome, brain malformations in children born to mothers infected during pregnancy, including encephalitis, myelitis, and microcephaly, have been identified.[12] Microcephaly is a disease involving impaired proliferation and death of cortical progenitors in the brain, which can lead to varying degrees of mental retardation.[13] The appearance of microcephaly[14] has made ZIKV neuropathy a focus of attention worldwide. Recent publications indicate that other emerging neurotropic flaviviruses may share the capacity for transplacental transmission with ZIKV, as well as the potential to infect growing fetuses and affect their development.[15] In this review, we describe progress in

Abbreviations used: CNS, central nervous system; DENV, dengue virus; GAG, glycosaminoglycan; hESC, human embryonic stem cell; hiPSC, human-induced pluripotent stem cell; HNPC, human neural progenitor cell; hNSC, human neural stem cell; IFN, interferon; JEV, Japanese encephalitis virus; NPC, neural progenitor cell; OPN, osteopontin; PAK4, p21-activated kinase 4; PMN, polymorphonuclear neutrophil; SOCS, suppressor of cytokine signalling; TLR, toll-like receptor; WNV, West Nile virus; YFV, yellow fever virus; ZIKV, Zika virus

TABLE 1 Mosquito-borne flavivirus classification

Cluster	Serocomplex	Virus
Mosquito-borne	Japanese encephalitis	West Nile
		Kunjin
		Japanese encephalitis
		Murray Valley encephalitis
		St. Louis encephalitis
	Dengue	Dengue-1
		Dengue-2
		Dengue-3
		Dengue-4
	None	Yellow fever

Selected serocomplexes (serological classification) and flaviviruses are shown in the table.

understanding neurologic diseases caused by several species of flaviviruses that can provide new directions for the study of neurological diseases and suggest mechanisms that underlie the neurotoxicity induced by ZIKV.

2 | POSSIBLE MECHANISMS BY WHICH FLAVIVIRUSES PENETRATE THE BLOOD-BRAIN BARRIER

The flaviviruses presented in this paper can produce neuropathy, with brain lesions being the most prominent neurologic effect. Research on the penetration of flaviviruses through the blood-brain barrier has increased. Notably, JEV[16] and DENV[17] have been found to cross the blood-brain barrier and cause encephalitis.

2.1 | Effects of inflammation

Inflammation and subsequent breaches in the blood-brain barrier play an important role in JEV invasion of the central nervous system (CNS).[18] Infection of microvascular pericytes in the brain can induce and/or amplify neuroinflammation caused by JEV infection.[19] Researchers have found that the inflammatory chemokine osteopontin (OPN) can loosen the blood-brain barrier in mice and promote WNV nerve invasive infection (Figure 1).[20] The study found that interferon (IFN) signalling regulates permeability of the blood-brain barrier after WNV infection.[21] Some researchers have found that WNV affects the activation of the JAK-STAT pathway by reducing the steady-state level of tyrosine phosphorylation, antagonizing the immune response induced by IFN (Figure 1).[22] Thus, we speculate that inflammation may assist the entry of flaviviruses and allow them to infect brain tissues.

2.2 | Apoptotic effects

High JEV loads have the potential to subvert host cell apoptosis by deactivating proapoptotic proteins.[23] In addition to causing inflammation, apoptosis damages barrier cells to increase the effects of inflammation, magnifying the resulting neuropathies.

2.3 | Gate theory

DENV destroys endothelial cells and forms vacuoles in brain tissue.[24] A number of researchers found that the virus activates endothelial cells and affects the structure and function of the blood-brain barrier, which promotes immune cell migration to benefit the virus.[17] The result is similar to the "gate theory" proposed by Andre Barkhordarian and other researchers. The "door theory" argues that DENV infection and immune activation open TH17/TH9-controlled gates and destroy close connections between endothelial cells that comprise the blood-brain barrier, so that immune cells and immune factors can penetrate the barrier,[25] causing inflammation and allowing the virus to enter the brain. To a certain extent, changes in the permeability of the blood-brain barrier have been shown to be closely related to the gate theory.

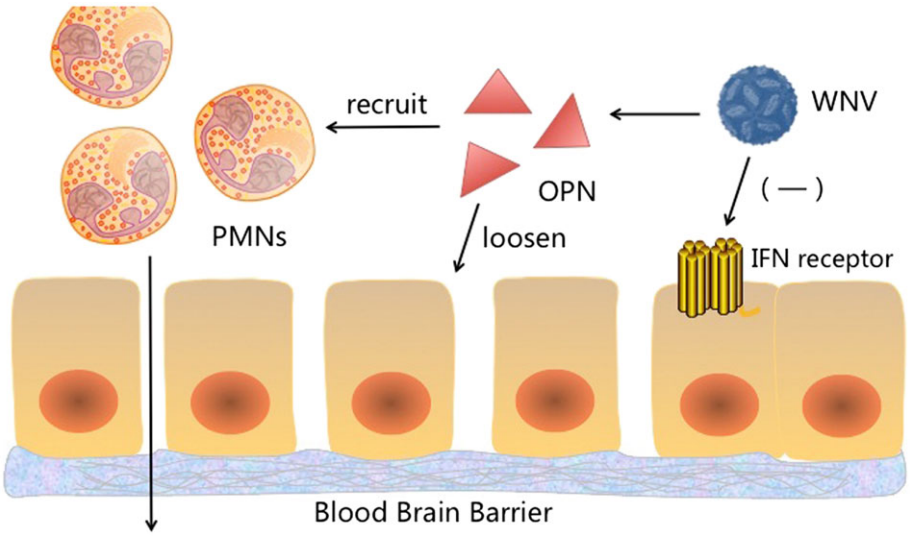

FIGURE 1 Schematic diagram of the extracellular effects of West Nile virus (WNV). WNV can induce the inflammatory chemokine osteopontin (OPN), which recruits polymorphonuclear neutrophils (PMNs), increasing the inflammatory effects (as shown on the left). WNV can also reduce the release of interferon (IFN), allowing the virus to penetrate the blood-brain barrier (shown on the right)

2.4 | Additional mechanisms

There may be additional mechanisms that assist WNV infection of endothelial cells or the choroid plexus epithelium and passively transport the virus to the CNS. WNV infects olfactory nerves and spreads to olfactory bulbs, entering through a "Trojan horse mechanism."[20] Infected immune cells transport the virus to the CNS, and infected neurons are relayed to other neurons by retrograde transport.[25]

Human trophoblasts constitutively release type III IFN, which protects trophoblast and nontrophoblast cells from ZIKV infection. However, this study could not determine whether ZIKV passes through the placenta through other means.[26] One study suggests that DENV, YFV, and ZIKV have similar tropisms for trophoblasts. ZIKV infection decreases the levels of IFN and increases the inflammatory immune response in trophoblasts; placental inflammation, in turn, leads to neonatal brain damage and the risk of premature birth.[27]

Therefore, research on barrier mechanisms is critical. Destruction of the barrier may result in a variety of injuries; therefore, the gate theory deserves further attention.

3 | NERVOUS SYSTEM TARGET CELLS INFECTED BY FLAVIVIRUSES

Flaviviruses infect human nervous system cells, causing neurological damage. Research on the target cells in the nervous system that are infected by flaviviruses helps us to understand the causes of neurological symptoms.

3.1 | Neural progenitor cells

JEV was found to suppress the cycling of neural progenitor cells (NPCs), preventing their proliferation.[28] Regarding the differentiation neural stem/progenitor cells (NSPCs), neuronal and astrocyte differentiation both appear to be severely affected.[29] Researchers have also found that DENV2 causes the death of NPCs and neurons, reducing brain volume.[30]

A recent study on ZIKV showed that human NPCs (hNPCs) show higher levels of infection (up to 90% higher) than three other viruses.[31] In addition, ZIKV infection causes cytopathic effect without affecting cell viability, decreasing the amount of proteins secreted in mice neural stem cell (NSC) supernatants.[32] This suggests that flavivirus infection may cause the depletion of neural precursors and/or the differentiation of NPCs, leading to neurological sequelae.

3.2 | Glial cells

Researchers have begun to study glial cells. White matter astrocytes have been found to be key responders to viral infection,[21,33] and ZIKV can infect astrocytes.[34] For JEV, microglia and astrocytes, especially the primary cell types, are the more important effector cell types.[35-41]

In addition, human microglia infected by JEV can transmit the virus to neighbouring cells in a contact-dependent manner.[42] Further, microglia have been found to devour ZIKV-infected NPCs and to transmit the virus to uninfected NPCs.[43] This is consistent with the previously mentioned Trojan horse mechanism.

In summary, the ability of microglia to spread and increase inflammatory effects also supports the infection of other cells.

3.3 | Immunocytes

In conjunction with these effects, $CD8^+$ T cells secrete proinflammatory cytokines and viruses lyse nerve cells, both of which contribute to the neuropathogenesis of WNV infection.[44] $CD8^+$ T cells may be involved in breakdown of the barrier system, making it easier for cells to cross the blood-brain barrier. These cells may also be involved in the exogenous damage of nerve cells.

3.4 | Cortical and neural cells

DENV2 can directly infect and replicate in neurons.[45] ZIKV was found to infect human embryonic stem cells (hESCs), human-induced pluripotent stem cells (hiPSCs), immature cortical neurons,[31] and human organoid cortical tissues.[46] This suggests that viruses might cause direct damage to neurons, directly leading to neurological symptoms.

Although DENV2 infection is similar to that of ZIKV, deleterious consequences of ZIKV infection in human NSCs (hNSCs), neurospheres, and brain organoids are not apparent.[47] This suggests that ZIKV may interfere with the proliferation of nerve cells, which may provide insight into the mechanisms of action of ZIKV.

4 | POSSIBLE MECHANISMS UNDERLYING FLAVIVIRUS-INDUCED NEURONAL LESIONS

Studying the possible mechanisms of neurocytopathies caused by flaviviruses will help clarify the mechanisms of flavivirus neurovirulence.

4.1 | Extracellular effect mechanisms

Extracellular effect mechanisms protect the body from damage and can help the virus to infect cells, aggravating the damage. Effector $CD8^+$ T cells[48] and IFN[49] play a protective role in viral infections and prevent nerve invasion by viruses, possibly maintaining the integrity of the blood-brain barrier. This extracellular effect may assist the virus in causing neuronal pathological changes, and the mechanism underlying this effect can be divided into the following components.

4.1.1 | Cell surface receptors

Researchers have identified "heat shock protein 70" (Hsp70).[50] One of these, GRP78,[51] is a possible receptor for JEV entry into cells (Figure 2). In addition, JEV may exploit dopamine-mediated neuronal communication to increase the susceptibility of D2R-expressing cells to JEV infection (Figure 2).[52] Currently, studies indicate that through a toll-like receptor 7 (TLR7)–related mechanism, pericytes might play a pathological role in Japanese encephalitis–associated neuroinflammation by initiating and/or amplifying inflammatory cytokine expression (Figure 2).[19] Glycosaminoglycans (GAGs), as JEV and ZIKV host

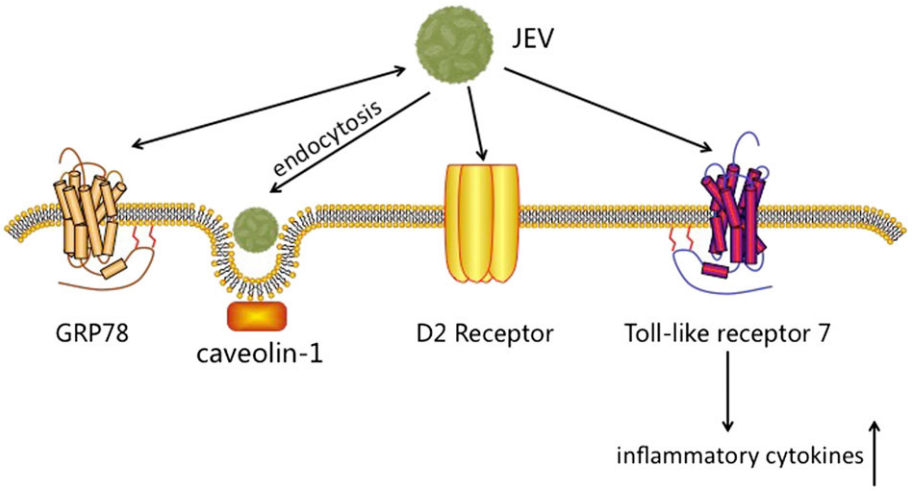

FIGURE 2 Mechanisms of Japanese encephalitis virus (JEV) entry. The figure illustrates the JEV cell surface receptors GRP78, D2, and TLV7, as well as caveolin-1–assisted entry of JEV into nerve cells

cell entry factors,[53,54] may facilitate the invasion of placental cells. This suggests that GAGs could have an important role to play in neuropathy. In addition, AXL is a candidate ZIKV entry receptor.[55]

Apart from the above receptors, JEV can invade human neuronal cells through a caveolin-1–dependent endocytosis pathway (Figure 2),[56] whereas DENV entry may be mediated by clathrin (Figure 3).[57] This suggests that flaviviruses have multiple routes to cell infection, rather than a single receptor that allows cell entry. Subsequent to entry, viral acidification-dependent viral replication within nerve cells leads to neurotoxicity.

4.1.2 | Cytokines and kinases

The absence of the chemokine receptor CCR5 gene[58] and receptor-interacting protein kinase (RIPK3)[59] enhances susceptibility of cells to viruses, resulting in increased risk of neuroinfectious diseases. DENV can increase the production of IFN-γ, IL-12, and CD80, activating microglial cells to serve as antigen-presenting cells, which in turn stimulated cytotoxic T lymphocyte (CTL) proliferation and activation (Figure 4).[60] These findings suggest that cytokines promote antiviral immune responses that limit infection and neuropathy.

Neurons in the brain are a potential source of proinflammatory cytokines able to mediate the activation of astrocytes.[61] However, a negative feedback loop also seems to contribute to neuropathy. Researchers have found that suppressor of cytokine signalling (SOCS) may play a role in neuroprotection, but limiting cytokine responses may enhance the ability of WNV and tick-borne encephalitis virus to spread and cause disease.[62] Cytokines have a dual nature that allows them to contribute to immunity to as well as to the pathogenicity of viruses. Defining the roles of cytokines can allow the construction of cytokine network maps and an understanding of the neuropathic mechanisms of flaviviruses.

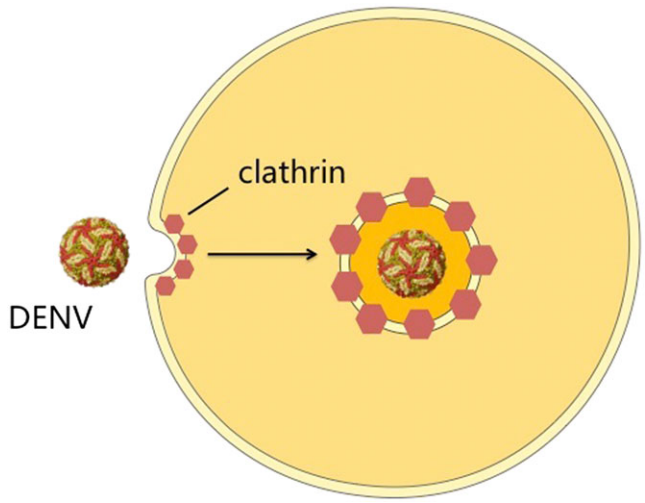

FIGURE 3 Dengue virus (DENV) endocytosis mediated by clathrin. Clathrin mediates the endocytosis of DENV, and the viruses subsequently replicate in the cell, leading to neurotoxicity

4.2 | Intracellular effect mechanisms

Understanding the mechanisms underlying intracellular effects is key to a comprehensive evaluation of neuropathic effects that will allow the development of antiviral therapies.

4.2.1 | Intracellular membrane structures

Neurons in the later phase of infection show structural changes in the endoplasmic reticulum (ER) and Golgi apparatus, as well as regenerative changes in membranous organelles.[63] The mitochondrial protein prohibitin and heterogeneous nuclear ribonucleoprotein hnRNPC (C1/C2) interact with viral RNA.[64] We hypothesize that flaviviruses bind to intracellular membrane structures, affecting their functions.

Studies have revealed that ZIKV binds to intracellular membrane structures (eg, mitochondria and vesicles).[47] Further, ZIKV particles have been detected in the nucleoplasmic compartment, and the nuclear membrane was disrupted in ZIKV infections (Figure 5), which has not been seen with DENV.[65] In addition, ZIKV capsid protein causes ribosomal stress.[66] Changes in intracellular membrane structures may be one mechanism by which ZIKV causes serious neuropathy.

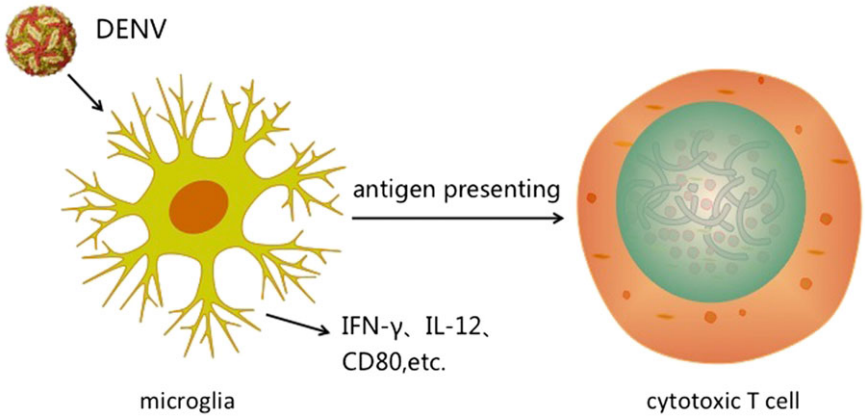

FIGURE 4 Dengue virus (DENV)–activated microglial cells. DENV can activate microglial cells to become antigen-presenting cells, which in turn stimulated cytotoxic T lymphocyte (CTL) proliferation and activation. IFN, interferon

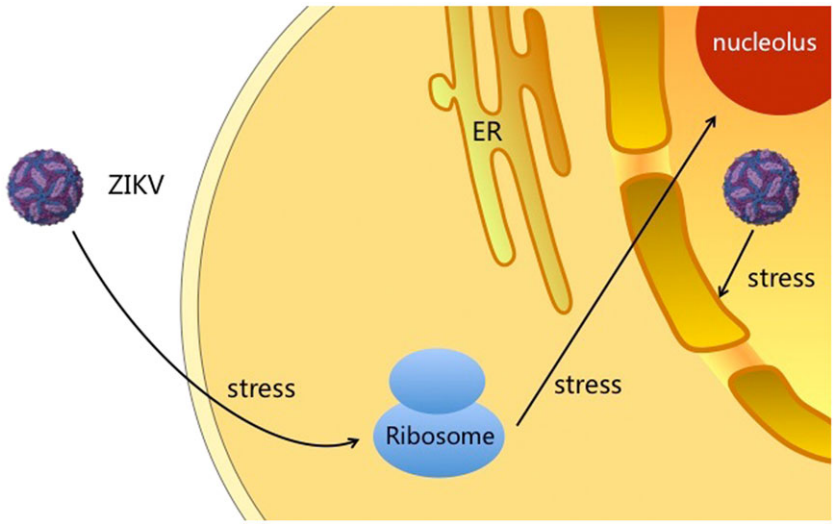

FIGURE 5 Intracellular Zika virus (ZIKV) infection leads to ribosomal stress and disruption of nuclear membranes. ZIKV damages nuclear membranes and mediates ribosomal stress, causing neuropathy. ER, endoplasmic reticulum

4.2.2 | Cell function

WNV may interfere with the intracellular protein degradation system,[67] and the release of S100B protein may enhance neurodegeneration.[68] Nedd4, an E3 ubiquitin ligase highly expressed in the CNS, attenuates JEV-induced autophagy, negatively regulating virus replication during infection.[69] JEV infection of neuronal cells leads to the activation of sensors of ER stress mediated by XBP1 and ATF6 (Figure 6).[70]

ZIKV infection drastically affects the host cell cytoskeleton.[71] In addition, infected NPCs can release infectious ZIKV particles, dysregulating cell cycle progression and increasing cell death.[72] Researchers have found that pTBK1 relocation may underlie neurodevelopmental defects associated with ZIKV infection.[73] A new report indicates that ZIKV kidnaps Musashi-1 (MSI1) for self-replication and disrupts the normal developmental progression of neural stem cells.[74]

These findings suggest that the virus uses various means to alter the function of cells and promote replication, which leads to nerve dysfunction. Changes in cell function may be linked to cytokines. Infected neurons may secrete proinflammatory cytokines[61] to promote inflammation and enhance neurotoxicity.

4.2.3 | Signalling pathways

By antagonizing postreceptor intracellular signalling of IFN, along with activation of SOCS3 expression and protein tyrosine phosphatase activity[75] in human astrocytes, p21-activated kinase 4 (PAK4) may regulate the JEV-mediated inflammatory response through the mitogen-activated protein kinase (MAPK) and NF-κB/AP-1 signalling pathways (Figure 6).[76] AXL regulates the expression of SOCS1[55] in a STAT1/STAT2-dependent manner, promoting ZIKV infection in human astrocytes.[34] Thus, it appears that flaviviruses not only antagonize the host IFN antiviral response but also utilize intracellular signalling mechanisms to support their pathogenesis.

4.2.4 | Apoptosis

The mechanisms involved in apoptosis induced by JEV include the following: direct neuronal infection, infection of other CNS cells such as microglia and astrocytes, and inflammation. The mechanisms involved have recently increased. ER stress reduces normal cellular activities[64] and mixed lineage kinase domain-like protein (MLKL)–mediated necroptosis,[77] leading to cell apoptosis.

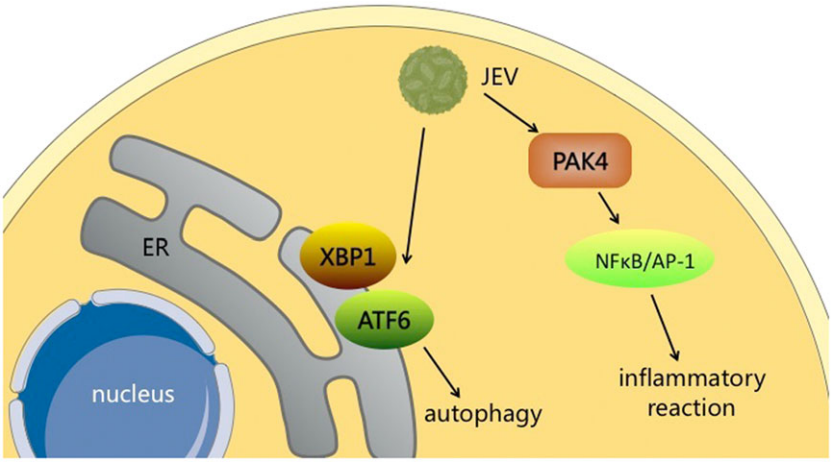

FIGURE 6 Japanese encephalitis virus (JEV) intracellular effect mechanism. JEV infection uses XBP1 and ATF6 stress sensors on the endoplasmic reticulum (ER) and the MAPK-NF-κB/AP-1 signalling pathway to support its pathogenesis

4.2.5 | Genes and proteins

Increased glutamate levels may be associated with oxidative stress.[78] A number of pathways may be responsible for JEV infection, including intracellular protein transport and ER stress–associated unfolded protein response.[79] Researchers have also found that 13 proteins are differentially expressed in JEV-infected and JEV-uninfected hNSCs (Table 2). Viral infection of cells leads to abnormal cell function, with abnormal proteins triggering relevant pathways.

To gain a deeper understanding of neuropathies, researchers have conducted genetic studies. DENV can raise the level of tumour necrosis factor α (TNF-α)[80] and expression of 151 genes, such as those encoding CD14, CD274, h2-ea, Tap1, Tap2, and Stat2.[81] Over 500 proteins and genes were differentially expressed with ZIKV infection.[82]

It is worth paying attention to how the virus induces programmed death, increasing the inflammatory effect.

TABLE 2 Thirteen proteins were found to be differentially expressed in the human neural stem cells (hNS1) cell line

Spot No.	Protein ID
1	Uncharacterized protein C19orf45 (Homo sapiens) NP_940936
2	Lamin isoform D (Homo sapiens) NP_001244303
3	Prohibitin (Homo sapiens) NP_001268425
4	Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit (Homo sapiens) NP_001677
5	Calreticulin precursor (Homo sapiens) NP_004334
6	78-kDa glucose-regulated protein precursor (Homo sapiens) NP_005338
7	Vimentin (Homo sapiens) NP_003371
8	Heterogeneous nuclear ribonucleoprotein C(C1/C2) (Homo sapiens) NP_001070911
9	Zinc finger protein 224 (Homo sapiens) NP_037530
10	RNA polymerase II subunit A C-terminal domain phosphatase (Homo sapiens) NP_430255
11	Actin, cytoplasmic 1 (Homo sapiens) NP_001092
12	HYOU1 protein (Homo sapiens) NP_001124463
13	Nebulin-related anchoring protein (Homo sapiens) NP_932326

A proteomic study of hNS1 cells after Japanese encephalitis virus (JEV) infection showed that 13 proteins were differentially expressed between infected and uninfected hNS1 cells.

5 | EFFECT OF FLAVIVIRUS GENE MUTAGENESIS ON NEUROTOXICITY

Many proteins in flaviviruses are involved in the pathogenesis of neuronal cells, such as E protein,[15,50,51] capsid protein-mediated ribosome stress,[34] and NS1 protein.[30,83] Here, we will describe the effects of various flavivirus gene mutations and their effects on nerve virulence.

5.1 | Mutations in the E protein gene

The Asn154 glycosylation site and its surrounding amino acid residues on the surface of ZIKV may contribute to attachment of the virus to host cells. Differences in amino acids of flaviviruses are associated with differences in virus recognition of and attachment to receptors on cells.[84,85] Glycosylation of E protein was the main determinant of a neuroinvasive phenotype.[53,86-88]

Further, revertant mutations at E107 and E138 of the E gene resulted in greater SA14-14-2 virulence.[89] With JEV, three substitutions in domain III[90] (305F → V, 326K → E, and 380R → T) showed that neither positions 380 (380T) nor 305 (305V) independently affected neuroinvasiveness, whereas residue 326 was found to be a critical determinant of YFV neuroinvasiveness.[91] The study also indicated that changes at position 303 may be important for YFV virulence.[92]

N-linked glycosylation of the E protein is an important determinant of ZIKV virulence and neuroinvasion[93] (Figure 9). Substitutions V603I and D679E were found in primary antigen region of the E protein. In addition, isoleucine at position 603 and glutamic acid at position 679 are present in all epidemic strains of ZIKV but in none of the pre-epidemic strains.[94] Taken together, we hypothesize that the E gene plays an important role in the neurotoxicity of flaviviruses.

5.2 | Mutations in the pre-M protein gene

One study has shown that determinants of flavivirus neuroinvasiveness are entirely located in the envelope proteins pre-M (prM)[95] and E[96] (Figure 7). A single amino acid substitution (serine to asparagine, S139N) (Figure 9) in the viral precursor membrane protein substantially increased ZIKV infectivity.[97] A V153M substitution

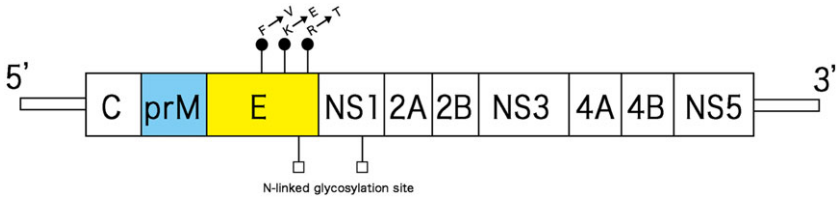

FIGURE 7 The locations of mutations in the yellow fever virus (YFV)–encoding gene. The pre-M (prM) and E proteins, coloured blue and yellow, respectively, may harbour determinants of neural invasion, with three possible causative substitutions in the E protein (305F → V, 326K → E, and 380R → T). In addition, *N*-linked glycosylation sites of E and NS1 proteins may affect neurotoxicity

in the region was found in all ZIKV epidemic strains. In addition, other amino acid substitutions, K143E, S139N, A148P, H157Y, V158I, K246R, and V262A, differed between the African and Asian lineages.[94] These results suggest that changes in the PrM protein gene affect flavivirus neurotoxicity.

5.3 | Mutations in nonstructural protein (NS) genes

The flavivirus NS1 glycoprotein is highly conserved and contains two *N*-linked glycosylation sites. The NS1 protein of WNV contains three *N*-chain glycosylation (n-x-s/T) sites in NS1(130), NS1(175), and NS1(207).[98] Researchers have found that mutants lacking the first or two glycosylation sites[88] (Figure 7) or substituting alanine in all three glycaemic sites with asparagine[98] can reduce the neuroaggression of WNV. Further, in addition to other residues in glycosylated sites NS1(130-132), mutation of asparagine to serine or glutamine can reduce the neuroinvasion and neurotoxicity of WNV in mice.[99] Other researchers have found that NS1-p250l mutations (Figure 8) are associated with significantly decreased neuroinvasive toxicity.[100] These studies indicate that both changes in glycosylation and mutations in the NS1 gene affect the neurotoxicity of flaviviruses.

The effects of wild-type NS2A on apoptosis induced without IFN have been verified.[101] In addition, nonstructural protein NS2A of WNV was found to be the main inhibitor of the IFN-β promoter, and an amino acid replacement in the NS2A gene (A30P, an alanine replaced with proline) (Figure 8) significantly reduced its inhibitory ability.[102] Further studies have shown that viral toxicity was reduced with the following substitutions (from high to low): WT > A30L > A30E > A30P/A30G.

There are four cysteine residues in NS4B (residues 102, 120, 227, and 237), and C102S mutations (Figure 8) are associated with attenuation of neuroinvasive and neurotoxic phenotypes.[103] Moreover, in NS4A gene, single point mutations to alanine and a deletion mutation of the viral protease dibasic cleavage site affect viral replication and reduce the formation of virus particles.[104]

In summary, mutations in NS1, NS2A, NS4A, and NS4B may affect the neurotoxicity of viruses.

5.4 | Other genes

Other substitutions in regions encoding proteins NS4A and NS5 and in the 3′ untranslated region (UTR)[90,94] may be virulence determinants in neuroadapted YFV strain YF17D.[105] This implicates the 3′ UTR in neurotoxicity.

Compared with the Uganda ZIKV prototype strain, five amino acid substitutions in the C protein (Figure 9) (N25S, L27F, R101K, I110V, and I113V), four in NS1 (E842D, K859R, A984V, and V1026I), and eight in NS3 and NS5 (M1970L, T2630 V, A2783V, N2892S, K3046R, P3158S, S3219D, and D3383N) are different between the pre-epidemic and epidemic strains. In addition, an analysis showed a possible recombination fragment in the NS2B coding region.[94] We can speculate that compared with earlier strains of the ZIKV, the prevalence of mutations in current ZIKV strains may explain why more

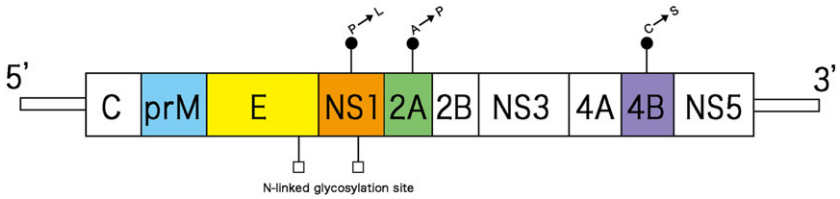

FIGURE 8 The locations of mutations in West Nile virus (WNV) gene encoding pre-M (prM), E, NS1, 2A, and 4B proteins. *N*-linked glycosylation sites in E and NS1 may be determinants of neural invasion. Substitutions in NS1(P250L), 2A(A30P), and 4B(C102S) are of particular interest

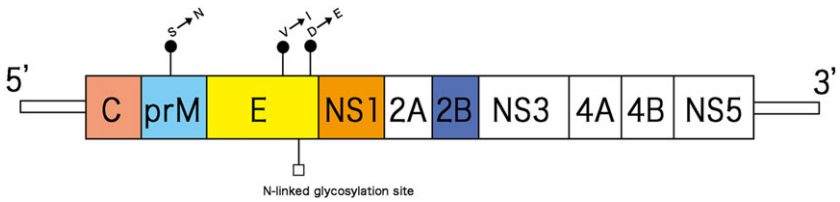

FIGURE 9 Locations of known mutations in Zika virus (ZIKV) genes encoding C, pre-M (prM), E, NS1, and N2B proteins. The coloured proteins may harbour determinants of neural invasion. Substitutions in prM (S139N) and E (V603I and D679E) may also affect neurotoxicity

recent ZIKV strains can penetrate the blood-brain and placental barriers, leading to brain lesions.

Flavivirus genes control the neurotoxicity and virulence of flaviviruses. Studying flavivirus genes and the interactions between virus genes and target cells will help us to understand the neurotoxicity associated with species gene expression and to systematically understand the neurotoxic effects of flaviviruses.

6 | HYPOTHESIS FOR ZIKV-INDUCED NEUROTOXICITY

In the neurotoxicity hypothesis proposed in this review, flaviviruses show differences in their neurovirulence. We propose possible mechanisms of intracellular effects that lead to ZIKV neurotoxicity. According to activation of the JAK-STAT pathway[22], the STAT2 gene[82] will

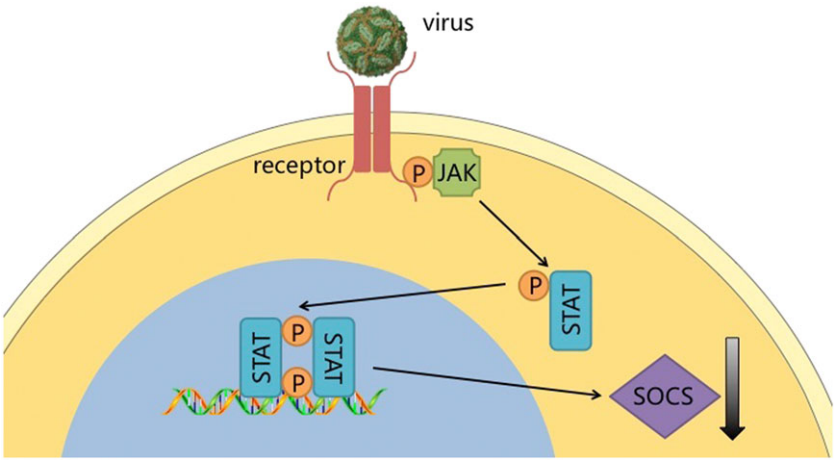

FIGURE 10 JAK-STAT pathway in Zika virus (ZIKV)–infected neurons. Sustained hyperactive JAK-STAT signalling and low suppressor of cytokine signalling (SOCS) expression may contribute to ZIKV neuropathy

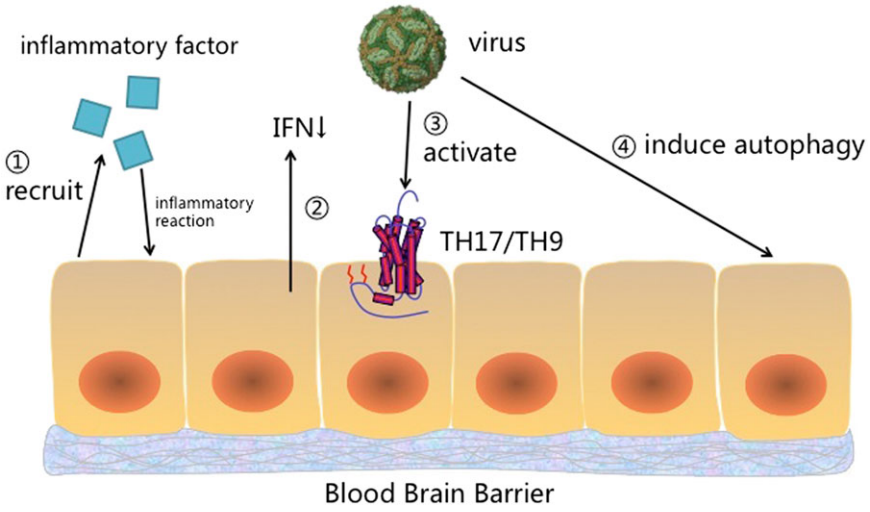

FIGURE 11 Illustration of a virus penetrating the blood-brain barrier. This virus can cause cell apoptosis, decrease inflammation, and open gates, allowing the virus to enter and damage nerves. IFN: interferon

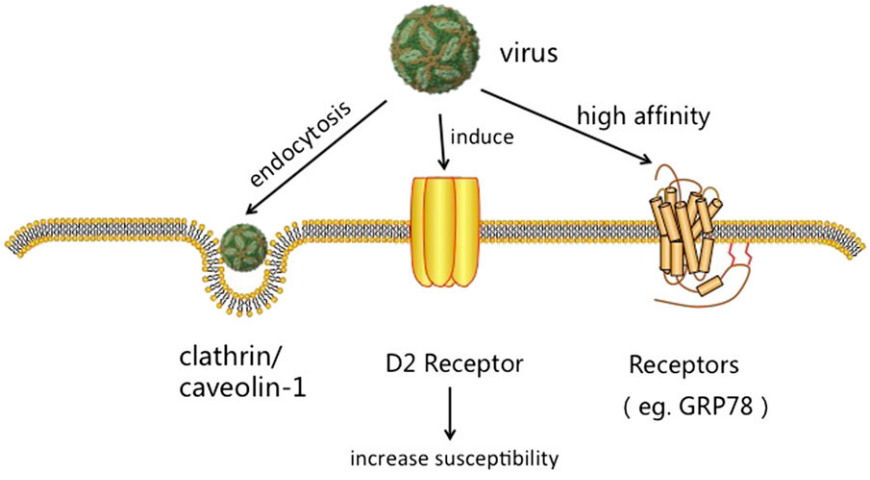

FIGURE 12 Flaviviruses in a nerve cell schematic. Flaviviruses may bind with high affinity to nerve cells, entering cells through multiple routes, increasing the sensitivity of nerve cells to infection

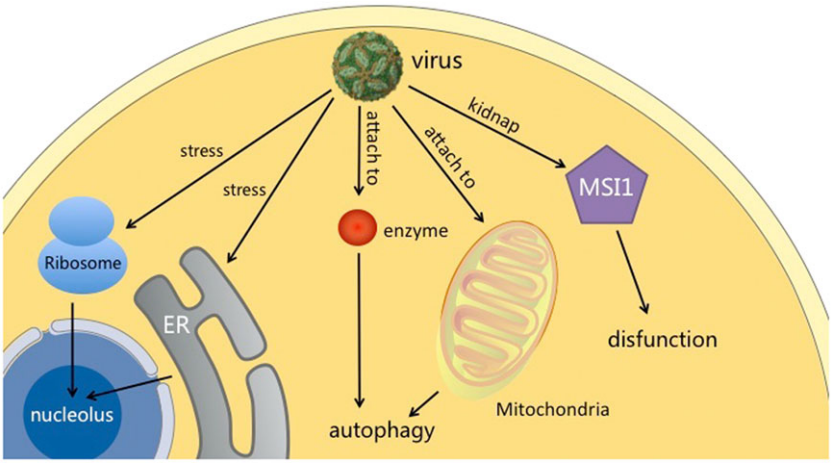

FIGURE 13 The cellular effect hypothesis. Flaviviruses may be linked cell membrane structure (mitochondria and vesicles), leading to ribosomal stress and so on eventually apoptosis. ER: endoplasmic reticulum

be upregulated and the STAT1/STAT2 pathway[35] will be activated. We speculate that the JAK-STAT signalling pathway plays a role in the effects observed in ZIKV-infected neurons.

ZIKV binds to nerve cell membrane receptors and deactivates the JAK-STAT pathway. STATs phosphorylated by JAK regulate the expression of cytokine genes such as IFN. The presence of ZIKV and type II IFN activate STAT2, resulting in sustained hyperactive JAK-STAT signalling and low SOCS expression. This eventually leads to abnormally active target cells and promotes the replication of ZIKV in these cells, resulting in neurotoxic lesions (Figure 10).

7 | SUMMARY

Flaviviruses can penetrate the blood-brain barrier through various means (Figure 11). For example, perivascular pericytes that are infected with flaviviruses recruit inflammatory cytokines and open TH17/TH9-controlled gates to increase the permeability of the blood-brain barrier. Paths to penetrate the blood-brain barrier are diverse, and flaviviruses are likely to have various mechanisms that lead to neuropathy.

Flaviviruses may enter nerve cells through various routes (Figure 12), including through high-affinity interactions with cell membrane receptors (eg, GRSP78) or cavolin. Following these interactions, cells are susceptible to flavivirus infection.

There are several explanations for neuropathic effects of flavivirus infection (Figure 13), such as the virus induces apoptosis and depletes cells of important proteins, altering normal cell function. There also may be a "Trojan horse mechanism."

We can draw on similarities of these viruses to better understand the mechanism of action of flaviviruses in the nervous system. With additional research, we will elucidate the mechanism of neurovirulence of flaviviruses to develop more effective antiviral drugs and optimize treatment of diseases associated with these viruses.

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CONFLICT OF INTEREST

The authors have no competing interest.

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