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REVIEW

Emerging viral infections: role of flavivirus NS1-mediated rewiring of PRR signaling

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ABSTRACT: Flaviviruses, including Dengue, West Nile, Zika, and Japanese encephalitis viruses, are arthropod-borne RNA viruses that pose an increasing global health threat. This review summarizes the role of nonstructural protein 1 (NS1), a multifunctional glycoprotein found in intracellular and secreted forms, as a key regulator of innate immunity. NS1 modulates several pattern recognition receptor pathways, including TLRs, RLRs, SR-B1-related mechanisms, and inflammasome platforms, thereby altering cytokine and interferon responses. Its effects are virus- and context-dependent. WNV NS1 inhibits TLR3/TRIF signaling, reducing IRF3 activation, type I interferon production, and interferon-stimulated gene expression. In contrast, DENV NS1 is linked to inflammatory signaling, particularly through TLR4. At the cytosolic level, NS1 from DENV, WNV, and ZIKV disrupts RIG-I/MDA5–MAVS signaling and weakens IFN-β induction. NS1 also affects inflammasome pathways: DENV promotes IL-1β release through a CD14-dependent mechanism, ZIKV suppresses cGAS-mediated antiviral signaling, and JEV promotes NLRP3 inflammasome assembly. Overall, NS1 selectively dampens interferon-mediated antiviral defenses while sustaining or enhancing inflammation, contributing to endothelial dysfunction, neuroinflammation, and severe disease.

KEYWORDS: Flavivirus, nonstructural protein 1 (NS1), pattern recognition receptor (PRR) signaling modulation, antiviral immunity, inflammation

1 Introduction

Innate immunity acts as the first defensive barrier against pathogens, providing rapid and non-specific protection through physical barriers (skin and mucosal epithelium) and chemical mechanisms such as antimicrobial enzymes, pH control and the complement system [1]. Its activation is initiated by the detection of pathogen-associated molecular patterns (PAMPs)—including viral DNA or RNA, lipopolysaccharide (LPS) and flagellin [2]—together with damage-associated molecular patterns (DAMPs) released by stressed or damaged cells (e.g., extracellular ATP, histones, mitochondrial DNA, HMGB1 and heat shock proteins) [3–5]. These signals are sensed by PRRs, which trigger intracellular cascades leading to the transcription of pro- and anti-inflammatory cytokines, thereby shaping early inflammation and antiviral defenses [6]. PRRs are commonly categorized into TLRs, C-type lectin receptors (CLRs), RLRs, NOD-like receptors (NLRs) and AIM2-like receptors (ALRs), differing in structural features and intracellular localization [7,8].

The purpose of this review is to clarify how flaviviral NS1 reshapes innate immune signaling by modulating PRR pathways, suppressing antiviral interferon responses, and promoting inflammatory mechanisms linked to severe disease.

2 Pattern Recognition Receptors (PRR) and Cytokine Signaling During Viral Infections

Viral infections pose a significant global health threat, initiating a rapid innate immune response primarily driven by PRRs that detect conserved PAMPs. These sensors, including TLRs on endosomal membranes and RLRs in the cytoplasm, recognize viral components to activate signaling cascades, leading to type I interferon (IFN-I) production, pro-inflammatory cytokines, and antiviral states that limit pathogen spread [9].

This PRR-mediated sensing is particularly critical for emerging RNA viruses, which often generate pandemics due to high mutation rates,

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zoonotic spillovers, and efficient airborne or vector-borne transmission. In infections like Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—an emerging positive-sense RNA coronavirus- and Flaviviruses (e.g., Dengue virus (DENV), Zika virus (ZIKV), West Nile virus (WNV)), early detection by cytoplasmic retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) distinguishes non-self RNA motifs—such as 5′-triphosphate ends or double-stranded RNA (dsRNA) intermediates—from host transcripts, enabling antiviral defenses despite their rapid replication and immune evasion strategies [10–12].

However, viral nonstructural proteins—particularly flaviviral NS1—also act as both targets and modulators of sensing pathways. For instance, secreted NS1 (sNS1) can interfere with TLR signaling and complement activation, while intracellular forms contribute to immune evasion by disrupting PRR activation, thereby promoting viral replication and exacerbating pathogenesis in infected hosts [13]. This dual interplay underscores the particular strategies employed by Flaviviruses to subvert host defenses, highlighting NS1 as a key therapeutic target in these emerging infections.

2.1 Membrane-Associated PRRs

Membrane-associated PRRs mainly include TLRs and CLRs, strategically positioned at the plasma membrane or within endosomal compartments to detect extracellular microbes or internalized pathogen-derived material [8,14]. Their activation similarly converges on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), mitogen-activated protein kinases (MAPK) and interferon regulatory factor (IRF)-dependent signaling, coupling pathogen sensing to inflammatory cytokine/chemokine induction and, particularly for endosomal receptors, to interferon-driven antiviral programs [15–18].

2.1.1 Cell Surface PRRs

Cell surface PRRs detect extracellular pathogens and microbial components exposed at the cell boundary. Plasma membrane TLRs and several CLRs expressed on innate immune cells (and also on epithelial/stromal sentinels) promote rapid inflammatory signaling, typically via NF-κB and MAPKs [8,14]. This results in robust production of pro-inflammatory cytokines and chemokines, amplifying inflammation and supporting immune cell recruitment and activation [15,17,18]. CLRs additionally contribute to endocytosis/phagocytosis and can modulate adaptive immunity, also through functional crosstalk with TLR pathways [19–23].

2.1.2 Endosomal PRRs

Endosomal PRRs are specialized in sensing nucleic acids derived from internalized pathogens, making this compartment particularly relevant for antiviral responses [8,14]. Endosomal TLR signaling prominently engages IRF-dependent pathways to induce IFN-I (IFN-α/β), thereby establishing an antiviral state [16], while NF-κB/MAPKs can concurrently sustain inflammatory cytokine and chemokine transcription [15,17,18]. This dual output positions endosomal PRRs as key determinants of the balance between protective antiviral immunity and inflammatory pathology.

2.2 Cytosolic PRRs

Cytosolic PRRs include RIG-I-like receptors (RLRs), NOD-like receptors (NLRs) and AIM2-like receptors (ALRs), which sense intracellular PAMPs/DAMPs and are pivotal for antiviral signaling and inflammatory amplification [8]. These pathways converge on transcription factors such as NF-κB, MAPKs and IRFs, coordinating cytokine and chemokine programs [6]. In particular, NF-κB drives the expression of pro-inflammatory cytokines (TNFs, IL-6, IL-1β), amplifying inflammation and recruiting effector cells [15], while IRF3 promotes IFN-I (IFN-α/β) to establish an antiviral state [16]. MAPKs (p38, JNK, ERK) further enhance chemokine secretion (e.g., CXCL8/IL-8 and CCL2), sustaining leukocyte recruitment to infected tissues [17,18]. In addition, several cytosolic PRRs—especially NLRs and ALRs—contribute to inflammasome assembly, enabling caspase-dependent processing of IL-1β and IL-18 and triggering pyroptotic cell death, which can aid pathogen clearance but may also exacerbate inflammation [6].

Several viral effectors, including viral proteins such as Flavivirus nonstructural protein 1 (NS1), exploit PRR localization and signaling hubs to reprogram cytokine outputs (*table 1*). Thus, during Flavivirus infection, the interaction of these receptors, especially TLRs, with NS1 represents a critical checkpoint at the interface between cellular sensing and antiviral and inflammatory responses.

Accordingly, the next section will address how flaviviral NS1 engages and reshapes PRR-dependent pathways. Despite the literature mainly focusing on PRR interaction with NS1 from WNV and Dengue DENV, we also summarize the available information concerning NS1 from other flaviviruses.

3 PRRs Involvement in Flavivirus NS1 Sensing

PRRs play a pivotal role in detecting Flavivirus NS1, contributing to the balance between innate immune sensing and the viral escape strategies associated with diseases like DENV, Zika (ZIKV), and WNV viruses [24,25]. Cytoplasmic sensors recognize dsRNA motifs from NS1-replication complexes to induce IFN-I responses, but NS1's RLR-mimicking structure might interfere with this process in order to limit antiviral defenses [26]. In fact, several studies reported that secreted sNS1 hexamers evade extracellular PRRs by binding complement factors and dampening TLR3 signaling, as intracellular dimeric NS1 protects infected cells from phagocytosis and modulates ER pathways [27,28].

The effect of NS1-TLRs interaction varies across studies and viral species, as demonstrated by conflicting evidence regarding its engagement with TLR4 and

Table 1
Summary of PRRs localization, main signaling pathways and cytokine outputs

PRR family	Localization and expression	Function	Signaling pathways	Cytokine output	References
Plasma membrane TLRs	Innate immune cells (macrophages, DCs, neutrophils); epithelial and stromal cells; plasma membrane	Recognition of extracellular viral components (structural proteins, envelope glycoproteins)	NF-kB, MAPKs	TNF-α, IL-6, IL-1β; chemokines (CXCL8, CCL2)	[15–18]
Endosomal TLRs	DCs, macrophages, B cells	Detection of internalized viral RNA/DNA	IRF3/IRF7; NF-kB; MAPKs	Type I IFNs (IFN-α, IFN-β); pro-inflammatory cytokines and chemokines	[14,16–18]
RLRs	Virus-permissive cells (epithelial cells, fibroblasts, immune cells); cytoplasm	Cytosolic sensing of viral RNA during replication	IRF3/IRF7; NF-kB	Type I IFNs (IFN-α, IFN-β); TNF-α, IL-6	[8,14,16]
NLRs	Myeloid and epithelial cells; cytoplasm	Intracellular PAMPs/DAMPs sensing; inflammasome activation	NF-kB; inflammasome assembly via caspase-1	IL-1β, IL-18	[6,8,14]
ALRs	Myeloid and epithelial cells; cytoplasm	Cytosolic host-derived DNA sensing during Flavivirus infection; inflammasome activation	Inflammasome assembly via caspase-1	IL-1β, IL-18	[6,8,14]

Note: Pattern Recognition Receptors (PRRs), Toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (RLRs; Retinoic acid-inducible gene I-like receptors), NOD-like receptors (NLRs; Nucleotide-binding oligomerization domain-like receptors), AIM2-like receptors (ALRs; Absent in melanoma 2-like receptors), Dendritic cells (DCs), B lymphocytes (B cells), Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Mitogen-activated protein kinases (MAPKs), Interferon regulatory factor 3 (IRF3), Interferon regulatory factor 7 (IRF7), Tumor necrosis factor alpha (TNF-α), Interleukin 6 (IL-6), Interleukin 1 beta (IL-1β), Interleukin 18 (IL-18), C-X-C motif chemokine ligand 8 (CXCL8), C-C motif chemokine ligand 2 (CCL2), Interferon alpha (IFN-α), Interferon beta (IFN-β), Type I interferons (Type I IFNs), Pathogen-associated molecular patterns (PAMPs), Damage-associated molecular patterns (DAMPs).

TLR2/6, as well as its inhibitory effects on TLR3 signaling [29–31]. Together, these interactions highlight NS1 as a multifunctional immunomodulator capable of promoting immune evasion or pathological inflammation.

3.1 NS1-TLR3 Interaction

Several studies investigated the TLR3 engagement by Flavivirus NS1 [29,32,33]. Notably, NS1 of WNV has been described to exert a strong inhibitory effect on TLR3-mediated signaling, which is essential for the recognition of viral dsRNA within endosomal compartments [34]. This viral protein interferes with the TLR3 pathway either through direct interaction with the receptor or by targeting downstream adaptor molecules, such as TIR-domain-containing adapter-inducing interferon-β (TRIF). This interference blocks the phosphorylation and nuclear translocation of IRF3 and also suppresses the NF-κB activation [32], supporting the antagonist role of NS1 against TLR3. The net result is a marked reduction of IFN-I secretion, particularly IFN-β, accompanied by diminished expression of pro-inflammatory cytokines, including IL-6, TNF-α and interferon-stimulated genes (ISGs) that normally establish an antiviral state [29] (*figure 1A*).

Attenuation of TLR3 stimulation has significant downstream consequences for host tissues. In endothelial cells, reduced IFN signaling, together with direct effects of secreted NS1 reported in related Flaviviruses such as DENV, leads to vascular barrier dysfunctions (*figure 1A*). This results in downregulation of tight junction proteins, including claudin-1 (CLD1) and occludin (OCLN), increased vascular permeability, and enhanced leukocyte transmigration [35,36].

Within the central nervous system (CNS), NS1-mediated suppression of TLR3 signaling limits microglial activation, the primary innate immune defense within the brain parenchyma [36]. Consequently, the production of chemokines, such as CXCL10, reactive oxygen species (ROS), and phagocytic activity toward infected neurons is reduced (*figure 1A*). This dampened neuroinflammatory environment paradoxically facilitates neurovirulence, while delaying the activation of effective adaptive immune responses [33,37].

Although direct evidence for a specific interaction between ZIKV NS1 and TLR3 is limited, TLR3-mediated signaling is recognized as an important component of the innate immune response during ZIKV infection. In neural cells and astrocytes, ZIKV infection activates TLR3 signaling, increasing pro-inflammatory cytokines such as IL-6, which can suppress interferon-mediated antiviral responses and facilitate viral replication [38]. While the direct role of NS1 in modulating TLR3 remains unconfirmed, NS1 proteins from related flaviviruses have been shown to interfere with TLR3-dependent pathways, suggesting that ZIKV NS1 may employ a similar immune-evasion strategy. Of note, direct evidence for DENV NS1 engagement with TLR3 is currently absent from the literature, representing a knowledge gap that future studies should address.

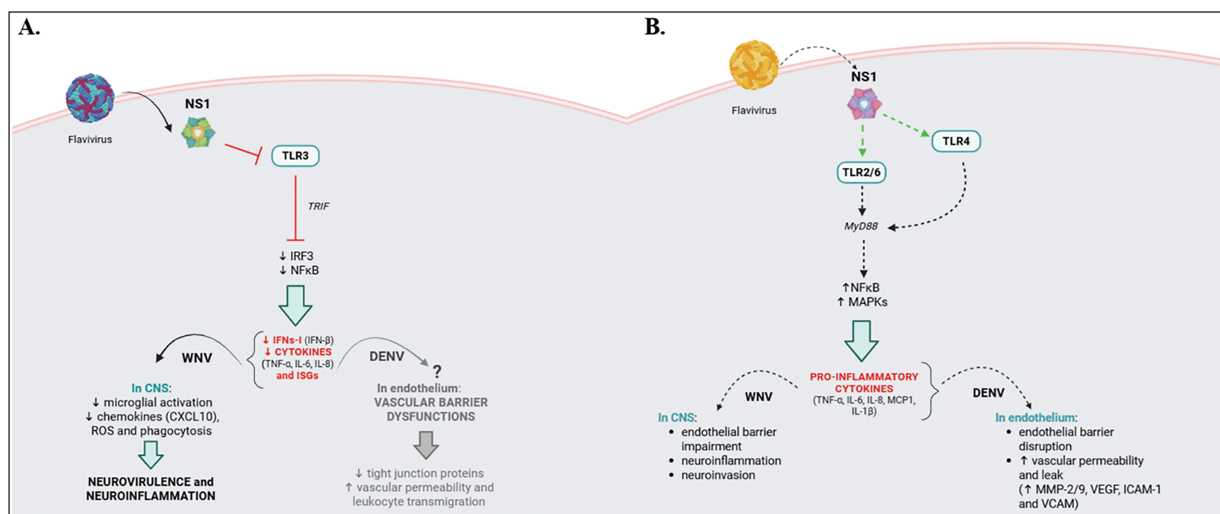

Figure 1: Schematic representation of WNV and DENV NS1 interaction with TLR3 (**A**), TLR4 and TLR2/6 pathways (**B**), and their effect on CNS and endothelium. Note: Nonstructural Protein 1 (NS1), Toll-Like Receptor 3 (TLR3), Toll-Like Receptor 2/6 (TLR2/6), Toll-Like Receptor 4 (TLR4), TIR-domain-containing adapter-inducing interferon-β (TRIF), Myeloid Differentiation Primary Response 88 (MyD88), Interferon Regulatory Factor 3 (IRF3), Nuclear Factor Kappa B (NF-κB), Mitogen-Activated Protein Kinases (MAPKs), Interferons (IFNs), Interferon Beta (IFN-β), Tumor Necrosis Factor Alpha (TNF-α), Interleukin 6 (IL-6), Interleukin 8 (IL-8), Interleukin 1 Beta (IL-1β), Monocyte Chemoattractant Protein 1 (MCP-1), Interferon-Stimulated Genes (ISGs), Reactive Oxygen Species (ROS), C-X-C Motif Chemokine Ligand 10 (CXCL10), Central Nervous System (CNS), West Nile Virus (WNV), Dengue Virus (DENV), Matrix Metalloproteinase 9 (MMP-9), Vascular Endothelial Growth Factor (VEGF), Intercellular Adhesion Molecule 1 (ICAM-1).

3.2 NS1-TLR4 Interaction

Although TLR3 antagonism by WNV NS1 is well established, evidence supporting a direct molecular interaction between WNV NS1 and TLR4 remains limited. TLR4 is primarily associated with recognition of bacterial LPS rather than viral components. However, NS1 from DENV has been shown to engage TLR4 on immune and endothelial cells [39]. This interaction activates MyD88-dependent signaling pathways, resulting in the activation of NF-κB and MAPKs, with the subsequent production of pro-inflammatory cytokines, including TNF-α, IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) (*figure 1B*). Notably, this signaling does not induce IFN-I, reflecting the inflammatory bias of MyD88-dependent pathways [39,40]. The resulting cellular environment contributes to endothelial barrier disruption, increased vascular permeability, and the vascular leak observed in severe Dengue disease [41–43].

Nevertheless, recent findings challenge the extent of NS1-mediated TLR4 activation. A study conducted by Vajjhala et al. reported that NS1, even at clinically relevant concentrations, fails to activate TLR4 and induces only modest endothelial barrier impairment when compared with the effects of TNF-α produced by infected macrophages [30] (*figure 1B*). This discrepancy likely reflects differences in experimental design, including the concentration and glycosylation state of recombinant NS1, the choice of cell model, and the sensitivity of the assays used to detect TLR4 activation. Critically, Vajjhala et al. employed clinically relevant NS1 concentrations and compared outcomes to TNF-α as a reference stimulus, whereas prior studies used higher supraphysiological concentrations [30]. Reconciling these results requires caution: while functional TLR4 engagement by DENV NS1 cannot be excluded, its contribution relative to indirect inflammatory mediators (e.g., TNF-α from infected macrophages) may be context- and concentration-dependent. In reviewing controversial data, we have chosen to present both lines of evidence while clearly flagging the uncertainty.

It has been hypothesized—though not experimentally demonstrated—that potential activation of TLR4 by WNV NS1 or its secreted form in microglial cells could exacerbate neuroinflammation through increased production of TNF-α and IL-1β, enhanced ROS generation, and induction of nitric oxide synthase, thereby creating a cytotoxic environment that compromises neuronal survival and blood–brain barrier integrity during WNV neuroinvasion. WNV NS1 has been detected at elevated levels in the brain during neuroinvasion [44], but a direct NS1–TLR4 interaction in microglia remains undemonstrated and must be regarded as speculative (*figure 1B*). Overall, current evidence indicates that while DENV NS1 clearly activates a proinflammatory TLR4 axis, its contribution to WNV pathogenesis remains uncertain and contrasts with NS1's inhibitory effects on TLR3 signaling.

Concerning other flaviviruses, direct evidence of ZIKV or JEV NS1 involvement in TLR4 signaling modulation is currently weak. However, structural analyses of their NS1 suggest interaction with host cell receptors, potentially influencing innate immune activation [25,45]. The direct activation of TLR4 by NS1 from these Flaviviruses has not been experimentally demonstrated, but the ability of proteins to modulate the TLR4 pathway increases the possibility that ZIKV and JEV may contribute to the regulation of pro-inflammatory responses. The role of NS1 in this part of immunity might have important implications for neuroinflammation and vascular integrity during infection, highlighting a potential role for NS1 in TLR4-mediated pathogenesis across these flaviviruses.

3.3 NS1-TLR2/TLR6 Interaction

TLR2/TLR6 heterodimers expressed on immune and endothelial cells are activated during DENV infection; however, as has been noted for TLR4, it is important to acknowledge that negative data also exist for this axis: Aguilar-Briseño et al. explicitly reported that recombinant NS1 alone failed to activate TLR2 in the absence of other viral components [46], indicating that the direct NS1–TLR2/TLR6 interaction may be context-dependent, strain-specific, or require co-factors present only during active infection. NS1 may nonetheless contribute to TLR2/TLR6 signaling within the broader infectious context, potentially through indirect mechanisms or synergy with other viral factors. Presenting this negative evidence alongside positive findings is essential for an accurate assessment of NS1's role as a PRR ligand. In the infectious context, TLR2/TLR6 activation may initiate MyD88-dependent signaling cascades that activate NF-κB and MAPKs, including p38 and c-Jun N-terminal kinase (JNK), leading to robust production of pro-inflammatory cytokines such as TNF-α, IL-6, IL-8, and MCP-1 [46] (*figure 1*). Similar to TLR4 signaling, IFN-I responses are largely absent due to the preferential induction of inflammatory rather than antiviral genes within MyD88-dependent pathways [31]. Although direct evidence for WNV NS1 interaction with TLR2/TLR6 remains limited, this pathway may contribute to cytokine amplification during flaviviral infections. Given these caveats, the capacity of NS1 to serve as a direct TLR2/TLR6 agonist should be considered unresolved.

In endothelial cells, infection-associated TLR2/TLR6 signaling, potentially influenced by NS1 or other viral factors, may exacerbate vascular barrier dysfunction by upregulating matrix metalloproteinases (MMP-2 and MMP-9), vascular endothelial growth factor (VEGF), and adhesion molecules, such as ICAM-1 and VCAM-1 (*figure 1*). These factors collectively promote degradation of tight junction proteins, including OCLN, CLD5, and ZO-1, resulting in increased paracellular permeability and facilitating plasma leakage and viral dissemination, as observed in Dengue hemorrhagic fever [25].

Within the CNS, analogous activation of TLR2/TLR6 signaling in microglial cells would be expected to intensify neuroinflammation through enhanced secretion of TNF-α, IL-1β, reactive oxygen and nitrogen species. While these responses may support clearance of infection, they also promote neuronal injury and further compromise blood–brain barrier integrity during WNV neuroinvasion (*figure 1*).

In the case of ZIKV and JEV, their NS1 interaction with TLR2 and TLR6 remains poorly understood compared with other flaviviruses. In ZIKV, NS1 can alter immune responses in microglia by inducing miR-146a, which suppresses NF-κB signaling and downstream cytokine expression, but this occurs without clear evidence of direct TLR2/TLR6 engagement [47]. In JEV infection, NS1 disrupts the blood–brain barrier via endothelial autophagy and MIF-mediated TLR4–NF-κB signaling, while microglial TLR2 activation during infection appears to result from viral infection rather than direct NS1 binding [48]. Although few studies have specifically addressed TLR2/TLR6 modulation by individual Flaviviruses, these findings nonetheless emphasize the context-dependent and multifaceted nature of NS1-mediated immunomodulation across distinct TLR pathways.

3.4 NS1-RIG I/MDA5 Interaction

Recent studies have demonstrated that both RIG-I and MDA5 can detect PAMPs produced during flaviviral infection, including those derived from DENV, JEV, and WNV [49,50].

In uninfected cells, RIG-I and MDA5 are maintained in an inactive state through phosphorylation and structural autoinhibition. After viral RNA detection, both receptors undergo conformational changes for downstream signal propagation. In particular, RIG-I activation involves interaction with mitochondrial antiviral-signaling protein (MAVS) [51]; by contrast, MDA5 recognizes long dsRNA and engages MAVS. Following activation, IRF3 and IRF7 are phosphorylated to stimulate the release of IFN-I, such as IFN-α/β. This results in the production of a wide range of pro-inflammatory cytokines such as IL-6, IL-8 and IL-10 [52].

Flaviviruses have evolved multiple strategies to avoid the activation of these pathways, with NS1 playing a central role. NS1 acts as a potent antagonist of RLR signaling by directly binding to RIG-I and MDA5, impairing their activation and promoting their degradation, which effectively suppresses MAVS-dependent signaling and the downstream production of IFNs-I (*figure 2*). NS1 also reduces TBK1 phosphorylation and blocks IRF3 activation, preventing its nuclear translocation and limiting the transcription of ISGs and pro-inflammatory cytokines [53]. Moreover, it is known that WNV NS1 is able to bind RIG-I and MDA5, leading to their proteasomal degradation and inhibition of K63-linked ubiquitination on RIG-I, which blocks IFN-β production [54] (*figure 2*). RLR (RIG-I/MDA5) suppression primarily reduces IRF3-dependent type I IFN/ISG induction, leading to an impaired antiviral state and enhanced viral replication. Endothelial glycocalyx disruption and vascular leakage are instead driven by extracellular NS1 activities and/or downstream inflammatory cytokines.

Moreover, NS1 is not the only flaviviral protein involved in immune evasion. NS2A, NS4B, and NS5 also inhibit RLR signaling and interferon responses by disrupting STAT1/STAT2 activity, suppressing IFN-I production and inhibiting RIG-I/MDA5 pathways [55,56]. Together, these proteins enable flaviviruses to evade host immunity and promote neuronal pathogenesis.

Recent evidence underscores the role of RIG-I–like receptors in microglial innate immunity. As the resident immune cells of the CNS, microglia rapidly sense tissue damage or infection, migrate to the affected area, and become activated, acquiring functions similar to macrophages and dendritic cells [57]. This includes the production of pro-inflammatory cytokines, such as TNF-α, IL-6, C-X-C motif CXCL10, CXCL1, CCL5, CCL3 and CCL2 [58]. Together with astrocytes, these neural cells express several PRRs, including

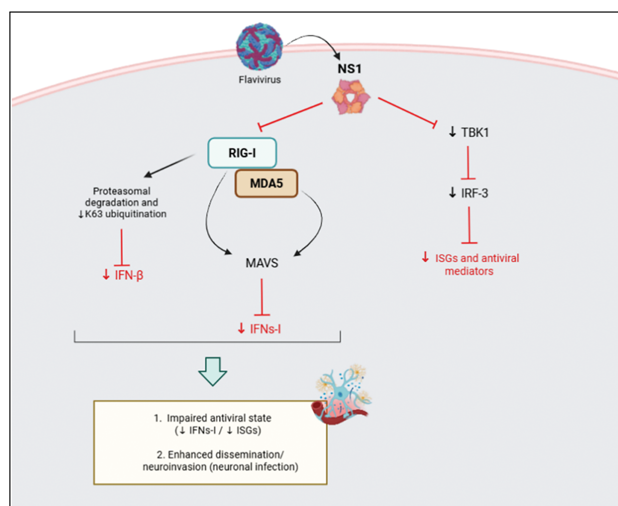

Figure 2: Schematic representation of RIG I/MDA5 pathway alterations by Flavivirus NS1. Note: Nonstructural protein 1 (NS1), Retinoic acid–inducible gene I (RIG-I), Melanoma differentiation–associated protein 5 (MDA5), Mitochondrial antiviral-signaling protein (MAVS), Interferon beta (IFN-β), Type I interferons (IFNs-I), TANK-binding kinase 1 (TBK1), Interferon regulatory factor 3 (IRF-3), Interferon-stimulated genes (ISGs).

endosomal TLRs (TLR3, TLR7, TLR8, and TLR9) and the cytosolic RNA sensors RIG-I and MDA5, which trigger IFN-I responses to viral RNA [59]. Because several Flaviviruses, including WNV, JEV, and ZIKV show marked neurotropism, modulation of host antiviral pathways is critical for CNS infection. Although RIG-I–like receptor expression and regulation in microglia are not fully defined, these sensors contribute to IFN-I-mediated antiviral defenses. Given the immunomodulatory properties of flaviviral NS1, interference with RLR signaling may represent an important mechanism of immune evasion that promotes viral persistence in the CNS.

Separately from RLR suppression, interest in RIG-I–like receptors has also increased, due to the ability of secreted NS1 from hemorrhagic Flaviviruses, such as DENV, to interact with the endothelium, disrupt the glycocalyx, and induce vascular leakage and hemorrhage [60]. Endothelial cells are a relevant target for flaviviral NS1 not only because of these structural effects, but also because they express RIG-I and MDA5, allowing them to sense viral RNA, particularly at the blood-brain barrier and in pulmonary endothelium [61]. In these cells, NS1 from DENV and ZIKV viruses has been shown to inhibit phosphorylation of TBK1, thereby suppressing IFN-β induction [25,53,62]. Although direct evidence of NS1 interactions with the endothelium remains limited, it is plausible to hypothesize that these interactions are significant, given the established role of RIG-I–like receptors in antiviral signaling and immune evasion. Further investigation is therefore required to elucidate the mechanisms and impact of NS1-mediated modulation of endothelial innate immunity.

3.5 NS1/SR-B1 Interaction

Unlike the TLR and RLR interactions discussed above, for which direct experimental evidence exists, the potential role of SR-B1 as a PRR-like entry point for flaviviral NS1 remains largely hypothetical. The following discussion is therefore grounded in indirect observations and structural plausibility rather than direct binding data and should be interpreted as a working hypothesis pending dedicated experimental validation. Although direct evidence for a role in viral infection remains limited, and current studies largely rely on indirect observations from related pathways, several lipid metabolism proteins have been proposed to participate in virus-host interactions as putative PRR. Among these, Scavenger receptor class B type 1 (SR-B1) has gained interest, because of its key role in cholesterol transport and its possible involvement in viral entry and replication [63].

SR-B1 is a member of the CD36 superfamily of membrane-bound glycoproteins, which includes CD36 and LIMPII [64]. It is widely expressed in the liver, adrenal glands, gonads, endothelial cells, and macrophages. SR-B1 plays a central role in reverse cholesterol transport (RCT) by mediating selective uptake of cholesteryl esters from high-density lipoproteins (HDL) into hepatocytes for bile acid production, and into steroidogenic tissues for hormone synthesis [65]. This process depletes cholesteryl esters from HDL particles, with SR-B1 sustaining the capacity of HDL to accept newly effluxed cholesterol from peripheral cells.

Beyond lipid transport, SR-B1 mediates immunomodulatory functions. HDL binding to SR-B1 activates Akt signaling and suppresses NF-kB activation in macrophages, promoting anti-inflammatory cytokine production, including IL-10 and TGF-β. Thus, SR-B1 integrates lipid metabolism with immune regulation [65].

Recently, SR-B1 has also emerged as a significant factor in viral pathogenesis. It serves as a principal entry receptor for hepatitis C virus (HCV) in hepatocytes and has been shown to facilitate ACE2-dependent entry of SARS-CoV-2 in various cell types [66,67]. Moreover, apolipoprotein A-I (ApoA-I), the main protein component of HDL, has been reported to bridge DENV particles to SR-B1, enhancing viral entry and infection [68]. These findings underscore the capacity of viruses to exploit host lipid transport pathways for cellular invasion.

The interaction between SR-B1 and flaviviral NS1 is of particular interest. In the case of DENV, NS1 is secreted as a glyco-lipoprotein complex containing triglycerides, cholesteryl esters, and phospholipids, closely resembling plasma HDL particles in composition [69]. Because NS1 must be internalized to exert its pathogenic functions, its lipoprotein-like structure may facilitate interaction with HDL receptors, such as SR-B1. Indeed, NS1 endocytosis has been shown to be required for NS1-mediated endothelial hyperpermeability, a hallmark of severe Dengue, with a major contributor to vascular leakage and hemorrhagic complications [70]. Such endothelial disruption can lead to systemic coagulation abnormalities and, in severe cases, intracerebral hemorrhage.

Importantly, SR-B1 is expressed on macrophages and microglia. In microglia, SR-B1 mediates binding

and endocytosis of fibrillar β-amyloid (fAβ), contributing to amyloid-associated cellular responses [71]. Recent evidence indicates that WNV NS1 can promote amyloid-β deposition and neurodegeneration through interference with TLR3 signaling. These observations raise the possibility that SR-B1-mediated internalization of flaviviral NS1 in microglia may represent an additional mechanism, linking viral infection to neuroinflammation and amyloid dysregulation [36].

Although direct experimental evidence for NS1-SR-B1 interactions in microglia remains limited, SR-B1's roles in lipid transport, viral entry and amyloid processing suggest that it may represent a critical element in Flavivirus-associated neuropathology. Given the speculative nature of current evidence, NS1–SR-B1 interaction should be considered a priority hypothesis for future mechanistic investigation.

Beyond its intracellular effects, secreted NS1 can interact with the SR-B1 on host cells, facilitating its uptake and modulating the immune response. Engagement of NS1 with SR-B1 has been associated with altered cytokine secretion and attenuation of IFNs-I signaling, demonstrating that NS1 can simultaneously influence both intracellular antiviral pathways and extracellular receptor-mediated responses [72]. Thus, NS1 can be considered a viral immune modulator, not just a RLR ligand, because it actively reshapes the cytokine output of the RLR pathway, specifically RIG-I and MDA5.

4 NS1 and Inflammasome

Inflammation is essential for antiviral defense but is also a major driver of Flavivirus-associated pathology, largely via pro-inflammatory cytokines produced downstream of inflammasome activation. NLRs are central mediators of inflammasome assembly [73], and the NLRP3 inflammasome is the best characterized platform: upon activation, NLRP3 recruits apoptosis-associated Speck-like protein containing a CARD (ASC) and pro-caspase-1, enabling caspase-1 maturation and subsequent processing of IL-1β and IL-18. IL-18 promotes IFN-γ production by NK and T cells, reinforcing Th1 immunity [74], whereas IL-1β amplifies inflammatory gene expression, lymphocyte recruitment and adaptive immune shaping [75,76]. Beyond cytokines, NLRP3 can induce caspase-1–dependent gasdermin D (GSDMD) cleavage and pyroptosis, although direct evidence for pyroptosis as a pathogenic determinant in flaviviral disease is still limited [77,78].

Across multiple Flaviviruses, NS1 emerges as a recurring inflammasome modulator. In DENV, NS1 induces caspase-1–dependent IL-1β release in murine and human macrophages without rapid pyroptotic death, with macrophages remaining viable while secreting IL-1β [79]. Mechanistically, NS1 drives cleavage of pro-IL-1β, GSDMD and pro-caspase-1 into their active forms in a dose-dependent manner, yet inflammasome activation unexpectedly occurs independently of NLRP3, since IL-1β processing persists in NLRP3-deficient BMDMs [80]. This phenotype led to the identification of a CD14 requirement: NS1 reduces surface CD14 (suggesting internalization), and IL-1β release, GSDMD cleavage and caspase-1 activation are abolished in CD14-deficient BMDMs, indicating CD14-dependent inflammasome triggering by NS1 [79]. It should be noted, however, that the molecular link between CD14 engagement and caspase-1 activation remains incompletely defined. By analogy with LPS-induced CD14 signaling, it has been hypothesized that NS1-bound CD14 undergoes internalization and activates an alternative inflammasome platform distinct from NLRP3, possibly involving non-canonical caspase-4/5 pathways or, alternatively, NLRP1; however, neither mechanism has been directly demonstrated in the context of NS1 signaling, and the pathway should therefore be regarded as partially characterized rather than fully resolved [78]. Importantly, comparable CD14-dependent, NLRP3-independent inflammasome activation has not yet been documented for WNV, ZIKV, or JEV NS1, representing a significant knowledge gap for future investigation.

In ZIKV, NS1 can enhance inflammasome output while simultaneously suppressing antiviral defense by recruiting USP8 to deubiquitinate and stabilize caspase-1, which then cleaves cGAS, dampening IFN-I signaling and promoting replication [81]. Consistently, NLRP3 deficiency is associated with increased IFNs-I and improved resistance *in vivo*; moreover, NLRP3 knockdown reduces ZIKV replication, whereas exogenous IL-1β does not, suggesting a proviral effect linked to caspase-1 activity rather than IL-1β per se [81]. Finally, JEV NS1 is implicated in NLRP3 assembly through stimulator of interferon genes (STING): phosphorylated STING is recruited to NS1-marked replication complexes, promoting local inflammasome assembly, with STING proton channel activity required for NLRP3 activation, IL-1β secretion and pyroptosis markers [82]. This fits with broader evidence that STING can interact with NLRP3, promote ER localization and support inflammasome activation [82].

Taken together, these observations identify NS1 as a key immunomodulatory factor that differentially regulates antiviral and inflammatory signaling among Flaviviruses (*table 2*), underscoring the importance of future investigations aimed at elucidating virus- and context-dependent mechanisms governing NS1-inflammasome interactions. Moreover, such studies may clarify the potential of NS1 as a therapeutic target for mitigating excessive and pathogenic inflammasome-driven inflammatory responses.

5 Future Perspectives: Therapeutic Targeting of NS1-PRR Interaction

Flaviviruses remain major global pathogens and, despite progress on antivirals targeting replication enzymes (e.g., NS3/NS5), clinical options are still limited. NS1 has emerged as an attractive complementary target because it combines a conserved structural scaffold with a dual role at the host–pathogen interface, acting both in replication-associated steps and—critically—during extracellular immune modulation. In this framework, future interventions can be conceptualized as direct (blocking NS1 itself and its pathogenic interfaces) or indirect (reducing NS1

Table 2
Flaviviral NS1 interactions with host PRRs and downstream effects

PRR family	PRR type	Cell type	NS1 Source	Main effect	Evidence	Reference
Endosomal TLRs	TLR3	Endothelial cells	WNV NS1	↓ IRF3/NF-κB; ↓ IFN-β, IL-6, TNF-α; ↑ permeability; ↓ tight junctions	Moderate: functional inhibition; suggested direct interaction; *in vitro*	[29,32]
		Microglia	WNV NS1	↓ microglial activation; ↓ CXCL10, ROS; impaired phagocytosis	Limited: functional, no direct binding	[33,37]
Plasma membrane TLRs	TLR4	Immune cells, endothelial cells	DENV NS1	↑ NF-κB/MAPK; ↑ TNF-α, IL-6, IL-8, MCP-1; vascular leak	High: functional assays; antagonists; *in vitro*	[39]
		CNS/brain context	WNV NS1	Elevated circulating NS1 associated with WNV brain spread; TLR4 involvement speculative	Correlative; no direct NS1–TLR4 activation demonstrated	[44]
	TLR2/TLR6 heterodimer	Immune cells, endothelial cells	DENV infection/Possible NS1 contribution	↑ NF-κB/MAPK; ↑ TNF-α, IL-6, IL-8, MCP-1; ↑ MMP-2/9, VEGF, ICAM-1/VCAM-1; ↑ permeability; direct NS1 agonism unresolved	Limited/controversial	[25,46]
RLRs	RIG-I/MDA5	Immune cells	DENV, WNV, JEV NS1	Direct binding; ↓ MAVS signaling, TBK1 phosphorylation; ↓ IFN-β; ↓ ISGs [60,61]	High: binding and degradation assays; functional inhibition; *in vitro*	[57]
		Microglia	WNV NS1			
		Endothelial cells	DENV, ZIKV NS1			
Scavenger receptors	SR-B1	Macrophages/ microglia	WNV NS1	NS1 uptake; endothelial hyperpermeability; altered cytokines; ↓ IFN-I signaling	Limited/hypothetical; uptake/endocytosis data, direct NS1–SR-B1 binding in relevant primary cells requires confirmation	[36]
		Endothelial cells	DENV NS1 (HDL-like secreted form)			[70]
NLRs	Inflammasome (CD14-dependent, NLRP3 independent)	Macrophages (murine and human)	DENV NS1	↑ Caspase-1 activation; ↑ IL-1β; GSDMD cleavage; no rapid pyroptosis	High: CD14 KO models, caspase assays	[79,80]
	NLRP3 inflammasome (via USP8-caspase 1 axis)	Macrophages	ZIKV NS1	↑ Caspase-1; ↑ IL-1β; ↓ IFN-I; proviral effect	High: genetic knockdown; *in vivo* association	[81]
	NLRP3-STING axis	Infected cells (JEV models)	JEV NS1	STING recruitment; ↑ NLRP3 assembly; ↑ IL-1β; pyroptosis markers	Moderate: mechanistic; STING required	[82]

Note: West Nile virus (WNV), Dengue virus (DENV), Zika virus (ZIKV), Japanese encephalitis virus (JEV), Pattern recognition receptors (PRRs), C-type lectin receptors (CLRs), Toll-like receptor 3 (TLR3), Toll-like receptor 4 (TLR4), Toll-like receptor 2 (TLR2), Toll-like receptor 6 (TLR6), RIG-I-like receptors (RLRs), Retinoic acid-inducible gene I (RIG-I), Melanoma differentiation-associated protein 5 (MDA5), NOD-like receptors (NLRs), Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Mitogen-activated protein kinase (MAPK), Interferon regulatory factor 3 (IRF3), Mitochondrial antiviral-signaling protein (MAVS), TANK-binding kinase 1 (TBK1), Stimulator of interferon genes (STING), Ubiquitin-specific protease 8 (USP8), Tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), Interleukin 8 (IL-8), Monocyte chemoattractant protein 1 (MCP-1), Chemokine (C-C motif) ligand 2 (CCL2), Chemokine (C-X-C motif) ligand 8 (CXCL8), Chemokine (C-X-C motif) ligand 10 (CXCL10), Interferon beta (IFN-β), Type I interferons (IFN-I), Interferon-stimulated genes (ISGs), Reactive oxygen species (ROS), Matrix metalloproteinase 2 (MMP-2), Matrix metalloproteinase 9 (MMP-9), Vascular endothelial growth factor (VEGF), Intercellular adhesion molecule 1 (ICAM-1), Vascular cell adhesion molecule 1 (VCAM-1), Scavenger receptor class B type 1 (SR-B1), Cluster of differentiation 14 (CD14), Gasdermin D (GSDMD), High-density lipoprotein (HDL), Knockout (KO).

availability/fitness or preventing its functional engagement). In both cases, a key expected benefit is the attenuation of NS1-driven rewiring of PRR signaling, limiting downstream NF-κB/IFN programs and inflammasome-linked inflammatory outputs triggered by NS1–PRR engagement.

5.1 Direct Therapies

Direct approaches aim to neutralize NS1 or mask its functional surfaces, thereby preventing NS1 binding to host targets and blunting downstream signaling events—including those initiated by NS1 interaction with membrane-associated PRRs (e.g., TLR axes) and

other PRR-linked inflammatory cascades. Structural conservation across Flaviviruses supports this strategy: despite ~40% amino acid identity, NS1 retains a conserved three-domain architecture (β-roll, wing, β-ladder) that offers druggable interfaces [83], while glycosylation is essential for NS1 maturation, secretion and virulence-related functions [84].

A first line of evidence comes from vaccine-induced functional neutralization: TAK-003 (Qdenga) elicits durable NS1-specific IgG responses with cross-reactivity, and post-vaccination sera can abrogate NS1-induced endothelial hyperpermeability and preserve glycocalyx integrity—supporting NS1 as a protective antigen concept [85,86]. Mechanistically, reducing NS1 bioavailability at the tissue interface is expected to dampen the upstream triggers that amplify PRR-dependent inflammation.

Even stronger mechanistic validation derives from monoclonal antibodies: broadly protective anti-NS1 mAbs prevent NS1-mediated endothelial dysfunction and confer *in vivo* protection independent of viremia, indicating that blocking NS1 "effector" functions can mitigate severe disease without necessarily suppressing replication [87]. Structural work highlights the β-ladder as a critical pathogenic interface, where blockade prevents endothelial binding and glycocalyx degradation [87]. This is directly relevant to NS1–PRR crosstalk because limiting NS1 docking/retention on host surfaces can reduce the probability and magnitude of PRR engagement and downstream cytokine escalation.

Finally, NS1-binding molecules/peptides provide a pharmacological counterpart to antibodies. The ApoA1-mimetic peptide 4F binds NS1, prevents endothelial interaction/internalization and blocks hyperpermeability across DENV, ZIKV and WNV; *in vivo* it provides dose-dependent protection in lethal DENV challenge models [88]. Importantly, these direct inhibitors target the extracellular pathogenic phase of NS1 activity—often more tightly linked to PRR-driven inflammatory pathology than viral load per se—and support the principle that functional NS1 neutralization can prevent vascular pathology independently of viral suppression [43,88].

In addition to antibody-mediated neutralization and NS1-binding peptides, a key direct strategy is to prevent secreted NS1 engagement with endothelial and immune cell surfaces, thereby limiting NS1–host receptor interactions that amplify PRR-linked inflammatory outputs. Secreted NS1 binds endothelium, activates complement, disrupts glycocalyx integrity and promotes vascular leak [40,89]. Accordingly, interventions that block NS1 docking/uptake—most prominently monoclonal antibodies targeting pathogenic NS1 epitopes—can functionally neutralize these extracellular effects and protect *in vivo* [90]. By preventing NS1 attachment and persistence at the tissue interface, these direct approaches are expected to reduce NS1-driven PRR activation and downstream cytokine amplification.

5.2 Indirect Therapies

Indirect approaches reduce NS1 pathogenic impact by limiting its production, maturation, or effective engagement with host surfaces, with the downstream goal of attenuating NS1-triggered PRR signaling and cytokine amplification once infection is underway.

The first route is replication-dependent suppression of NS1. Because NS1 expression depends on viral RNA replication, polymerase inhibition should theoretically reduce NS1 antigenemia; however, clinical evidence suggests this is highly time sensitive [91]. Balapiravir did not reduce viremia or NS1 antigenemia in a randomized trial, and ivermectin accelerated NS1 clearance without improving clinical outcomes [92]. These data support the idea that, by symptomatic presentation, NS1-driven cascades (endothelial damage and immune activation) may already be initiated [93], meaning PRR rewiring may persist even if replication is subsequently curtailed.

A second route targets ER-dependent folding and glycosylation, which are essential for NS1 maturation and secretion. Inhibition of ER α-glucosidases (e.g., celgosivir/castanospermine) disrupts viral glycoprotein processing and can impair NS1 maturation [94]. Since correct NS1 glycosylation is required for secretion and for glycocalyx-disrupting activity [60], interfering with this step is expected to reduce the pool of secretion-competent NS1 available to engage host receptors and trigger PRR-associated inflammatory outputs. Clinical translation, however, remains constrained by toxicity concerns and narrow therapeutic windows.

Collectively, indirect strategies are best suited to reduce the pool of secretion-competent NS1 through replication blockade or impaired ER maturation [91–94], whereas functional neutralization and attachment-blocking should be considered direct NS1-targeting interventions.

Thus, future efforts should focus on therapeutics that functionally neutralize NS1 or prevent its engagement with host tissues. This could include next-generation antibodies, peptides, or small molecules designed to block NS1 binding to endothelium or immune cells, thereby reducing PRRs activation and inflammation. Targeting NS1 at the extracellular interface might reduce clinical consequences of viral replication, such as vascular and inflammatory pathologies. Further, structural studies on the protein may guide the design of broad-spectrum inhibitors, enhancing cross-protection and improving clinical outcomes in future outbreaks.

6 Conclusions

This review presents a framework in which flaviviral NS1 functions as a central immunoregulatory determinant at the host–pathogen interface, exerting receptor-specific effects that modulate the quality and outcome of innate immune responses. Rather than acting only as a replication-associated cofactor, NS1 triggers multiple PRR systems and associated signaling, thereby influencing both antiviral response and inflammatory pathology. Among all TLRs, NS1 acts as a context-specific regulator of innate immunity, extending its modulatory role to RLRs and NLRs, attenuating

interferon-driven antiviral defenses while differentially influencing pro-inflammatory signaling cascades.

NS1 modulates innate immunity through multiple pattern-recognition receptor pathways, balancing suppression of antiviral defenses with promotion of inflammatory responses. On the TLR axis, NS1 can inhibit antiviral signaling, such as TLR3-mediated interferon production, while DENV NS1 has been implicated in TLR4-driven inflammatory responses and may contribute to TLR2/TLR6-associated cytokine release in the broader context of infection. NS1 also suppresses cytosolic RLR pathways, weakening interferon responses in microglia and endothelial cells, which facilitates viral persistence and spread. Beyond TLRs and RLRs, NS1 influences inflammasome activation, enhancing IL-1β production and inflammatory signaling while still dampening antiviral activity. This selective modulation of immune pathways promotes endothelial dysfunction, neuroinflammation, and tissue damage, with effects that vary depending on the virus, cell type, and context.

In conclusion, these findings support the idea that NS1 acts as a versatile regulator of innate immunity, exerting different effects across TLR, RLR and NLR signaling. Rather than broadly inhibiting host defenses, NS1 selectively modulates receptor-mediated pathways, weakening interferon-dependent antiviral responses while maintaining or enhancing inflammatory signaling that promotes viral persistence and spread. The variability observed among different flaviviruses and across tissue types underscores the need for precise, cell-type specific investigations of NS1-receptor interaction. Importantly, NS1 shares a conserved three-domain architecture (β-roll, wing, β-ladder) across flaviviruses despite only approximately 40% amino acid identity (with higher similarity values when conservative substitutions are considered), and its distinct glycosylation patterns further highlight it as a promising target for therapeutic intervention. Strategies that block NS1 activity, prevent its engagement with host cells, promote its degradation, or disrupt glycosylation patterns that are essential for its function could help restore balanced innate immune responses, mitigate vascular and inflammatory pathology, and reduce disease severity. Such approaches hold promise for broad-spectrum antivirals capable of improving clinical outcomes in future flavivirus outbreaks.

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