2009

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JOURNAL OF INTERFERON & CYTOKINE RESEARCH
Volume 29, Number 9, 2009

DOI: 10.1089/jir.2009.0069

REVIEW

Mechanisms of Evasion of the Type I Interferon Antiviral Response by Flaviviruses

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Virus survival and the ability to cause disease in mammalian hosts depend on their ability to avoid recognition and control by the interferon signal transduction and effector pathways. Flaviviruses comprise a large family of nonsegmented positive sense enveloped cytoplasmic RNA viruses, many of which are globally important human pathogens. Although the mechanistic details are still being dissected, new insight has emerged as to how a flavivirus minimizes the antiviral activity of type I interferon (IFN) to establish productive and potentially lethal infection. This review will summarize our current understanding of how mammalian cells recognize flaviviruses to induce an inhibitory IFN response and the countermeasures this group of viruses has evolved to antagonize this response.

Introduction

Flaviviruses comprise a genus of greater than 70 enveloped, positive sense RNA viruses and are distantly related to other Flaviviridae family members including hepatitis C virus (Lindenbach and Rice 2001). Many flavivirus infections are transmitted through the bite of an infected mosquito or tick, and have the potential to cause severe diseases in humans. Among the more common pathogenic flaviviruses in humans are Dengue (DENV), yellow fever (YFV), West Nile (WNV), Japanese encephalitis (JEV), Murray valley encephalitis (MVEV), Saint Louis encephalitis (SLEV), and tick-borne encephalitis (TBEV) viruses.

The ~11 kb flavivirus genome is transcribed as a single polyprotein and is cleaved by host and viral proteases into 3 structural and 7 nonstructural proteins. The structural proteins include a capsid protein (C) that binds viral RNA, a premembrane (prM) protein that blocks premature viral fusion, and an envelope (E) protein that mediates viral attachment, membrane fusion, and virion assembly (Mukhopadhyay and others 2005). The nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A NS4B, and NS5) regulate viral translation, transcription, and replication and also attenuate host antiviral responses. NS1 has cofactor activity for the viral replicase (Lindenbach and Rice 1997; Khromykh and others 1999), is secreted from infected cells (Flamand and others 1992; Flamand and others 1999), and antagonizes complement activation (Chung and others 2006). NS3 has protease, NTPase, and helicase activities (Murthy and others 2000; Xu and others 2005) with NS2B serving as a required cofactor for NS3 protease activity (Yusof and others 2000). NS4A and NS4B are small hydrophobic proteins that lack conserved sequence motifs of known enzymes. Overexpression of NS4A induces membrane rearrangements that are observed in flavivirus-infected cells (Roosendaal and others 2006; Miller and others 2007) whereas NS4B, along with NS2A, colocalizes with replication complexes (Mackenzie and others 1998; Miller and others 2006). NS5 encodes the RNA-dependent RNA polymerase and a methyltransferase (Egloff and others 2002; Malet and others 2007; Yap and others 2007).

After binding to poorly characterized cell surface receptors on mammalian cells, internalization of flaviviruses occurs through receptor-mediated, clathrin-dependent endocytosis (Gollins and Porterfield 1986a; Kimura and others 1986; van der Schaar and others 2007; Acosta and others 2008; van der Schaar and others 2008), possibly in cholesterol-rich microdomains (Medigeshi and others 2008). After trafficking to Rab5- and/or Rab7-positive endosomes (Krishnan and others 2007; van der Schaar and others 2008), a low pH-catalyzed structural change in the E protein (Bressanelli and others 2004; Modis and others 2004) facilitates viral fusion and release of the infectious genomic RNA into the cytoplasm (Gollins Porterfield 1986b). Flavivirus RNA traffics to the rough endoplasmic reticulum (ER) where it is translated, and serves as a template for a negative strand RNA

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intermediate that primes synthesis of positive strand viral RNA containing an N^7-methyl-guanosine cap but lacking a poly-A tail (Lindenbach and Rice 2001; Brinton 2002). Flavivirus positive strand RNA is either packaged within progeny virion or used to translate additional viral proteins. Flaviviruses assemble at and bud into the ER to form immature particles that display the prM protein. Following transport through the trans-Golgi network, furin-mediated cleavage of prM to M generates mature, infectious virions that are released by exocytosis (Guirakhoo and others 1991; Elshuber and others 2003).

Recognition of flaviviruses by host sensors

Interferon (IFN) responses are an initial and essential host defense program against many viruses, including flaviviruses. IFNs are produced during the earliest stages of viral infection after recognition of pathogen-associated molecular patterns (PAMP) by specific pathogen recognition receptors (PRR). In mammalian cells, the host detects and responds to infection by flaviviruses by primarily recognizing viral RNA through several distinct PRR including the cell surface and endosomal RNA sensors Toll-like receptors 3 and 7 (TLR3 and TLR7), and the cytoplasmic RNA sensors retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) (Fig. 1A and 1B). Binding of single- and/or double-stranded viral RNA to these PRR results in downstream activation of transcription factors, such as interferon regulatory factors 3 and 7 (IRF-3 and IRF-7) and NF-κB, and induction of IFN-α and -β. Secretion of IFNs followed by engagement of the IFN-αβ receptor (IFNAR) in an autocrine and paracrine fashion activates JAK-STAT-dependent and -independent signal transduction cascades (Stark and others 1998; Li and others 2007) that induce the expression of hundreds of interferon-stimulated genes (ISGs), a subset of which likely have antiviral activity against flaviviruses (Fig. 2).

Recent studies suggest that RIG-I and MDA5 contribute to the induction of host IFN and antiviral response to flaviviruses. Murine embryonic fibroblasts (MEF) deficient in RIG-I and MDA5 demonstrate decreased IRF-3 activation, delayed induction of host interferon and ISG responses, and augmented WNV and DENV replication (Fredericksen and others 2004; Fredericksen and Gale 2006; Fredericksen and others 2008; Loo and others 2008). In these cells, RIG-I appeared to prime the early IFN response whereas MDA5 has a more significant role in a second phase of IFN-dependent gene expression that occurs later in the course of infection (Fredericksen and others 2008). A genetic deficiency of IPS-1 (also known as Cardif, MAVS, or VISA), an essential RIG-I and MDA5 adaptor molecule that is anchored to the outer leaflet of the mitochondria, completely disabled the innate IFN response to WNV (Fredericksen and others 2008). However, MDA5 may be less essential for recognition of flaviviruses in some myeloid cell types, as IFN production by $MDA5^{-/-}$ myeloid dendritic cells remains largely intact after WNV infection (Gitlin and others 2006), and a deficiency of MDA5 in mice did not affect survival after JEV (Kato and others 2006). Consistent with this, JEV and DENV induce the host type I IFN response through a mechanism involving RIG-I/IRF-3 and NF-κB (Chang and others 2006).

Despite the compelling data from MEF suggesting that RIG-I and likely MDA5 recognize WNV RNA and induce type I IFN responses (Fredericksen and others 2008), IFN-α and -β production in mice appears largely independent of the downstream transcription factor IRF-3 (Bourne and others 2007; Daffis and others 2007). Individual cell types (myeloid, fibroblast, and neuronal) use distinct IRF-3 responses to protect against WNV infection through both IFN-dependent and -independent pathways (Daffis and others 2007). In cells that generate robust IFN responses after WNV infection in the absence of IRF-3, it is likely that alternate sets of PRR and transcriptional regulators are used.

TLR3, which is expressed on the surface of fibroblasts and in the endosomes of myeloid cells, promotes IRF-3 phosphorylation after binding double-stranded viral RNA through a complex signaling cascade that includes recruitment of TRIF and activation of the kinases TBK1 and IKK-ε (Matsumoto and others 2004; Schroder and Bowie 2005). Initial studies with TRIF-deficient MEF suggested that TLR3 may be dispensable for recognition of flaviviruses in cells (Fredericksen and Gale 2006). Indeed, $TLR3^{-/-}$ mice injected by an intraperitoneal route paradoxically showed decreased lethality despite higher peripheral viral titers, presumably because of blunted cytokine responses (eg, TNF-α) that normally facilitates WNV entry into the CNS (Wang and others 2004). Subsequent studies with $TLR3^{-/-}$ mice and a different North American WNV strain have shown increased viral burden in the brain and enhanced lethality (Daffis and others 2008a), as might be anticipated for a PRR that triggers a protective host immune response. *Ex vivo* and *in vivo* experiments suggest a cell-specific role of TLR3 as it protects against WNV largely by restricting replication in neurons.

TLR7 is an endosomal PRR that detects guanosine- and uridine-rich single-stranded RNA (Diebold and others 2004; Heil and others 2004) and activates IRF-7 via the Myd88 adaptor molecule. IRF-7 was identified as a primary regulator of antiviral gene induction after YFV infection (Gaucher and others 2008), with some of this activation occurring through TLR7 recognition of viral RNA (Querec and others 2006). Similarly, DENV stimulates IFN production in plasmacytoid dendritic cells in a TLR7-dependent manner after virus uncoating (Wang and others 2006). The antiviral IFN-α response against WNV is primarily mediated by IRF-7, and at least some of this signal is likely attributed to recognition of viral RNA by TLR7 (Daffis and others 2008b). An independent role for dsRNA-dependent protein kinase R (PKR) in the early induction of IFN in fibroblasts after WNV infection has also been observed (Gilfoy and Mason 2007).

IFN-mediated control of flaviviruses

Type I IFN is an important innate immune system regulator of viral infections (reviewed in Platanias and others (1996); Platanias (2005)). IFN-α and -β are secreted by many cell types following virus infection and induce an antiviral state by up-regulating genes with both direct and indirect antiviral functions. Type I IFN also primes adaptive immune responses through stimulation of dendritic cells, activation of B and T cells, and by preventing death of recently activated T cells (Stetson and Medzhitov 2006; Purtha and others 2008). Pretreatment of cells with IFN-α/β inhibits flavivirus replication *in vitro* (Diamond and others 2000; Anderson and Rahal 2002; Lin and others 2004; Best and others 2005; Samuel and others 2006), but treatment after infection is much less effective (Diamond and others 2000; Anderson and Rahal 2002; Crance and others

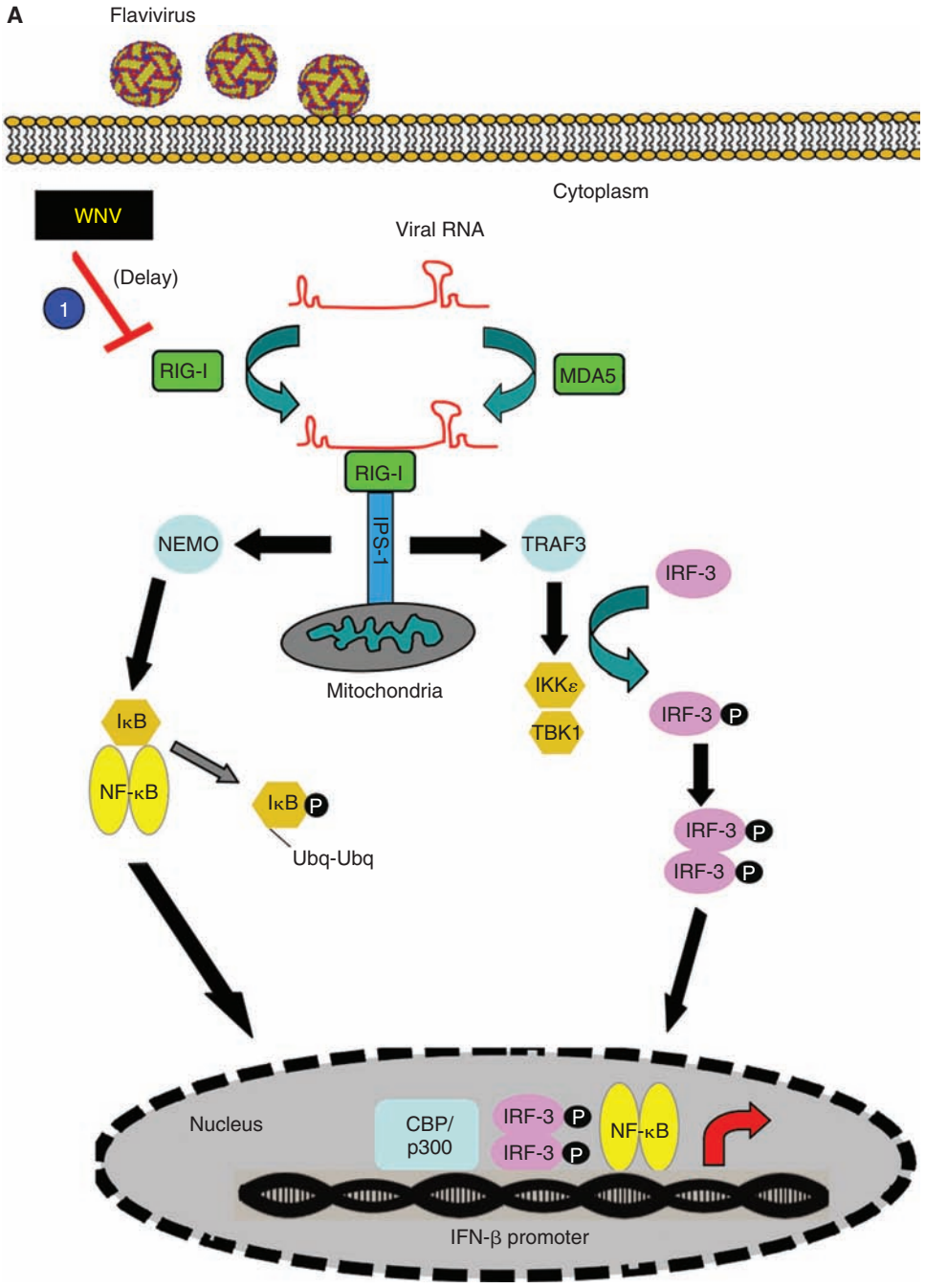

FIG. 1. Detection of flavivirus RNA by pathogen recognition receptors (PRRs) and mechanisms of viral evasion. (**A**) Cytoplamsic PRR and signaling cascade. Infection by flaviviruses produces dsRNA replication intermediates within the cytoplasm that display motifs recognized by the RIG-I and MDA5 helicases. Binding of viral RNA promotes an interaction with IPS-1 that results in recruitment of signaling proteins (NEMO and TRAF3) that activate IRF-3 and NF-κB. These transcription factors translocate to the nucleus and bind to the promoter region of the IFN-β gene leading to transcription and translation. (*Continued*)

2003; Samuel and Diamond 2005). Although flaviviruses can antagonize IFN-induced responses after infection (see below), IFN still restricts replication and spread *in vivo*. Mice lacking the type I IFN receptor ($IFNAR^{-/-}$) show markedly enhanced lethality and replication after infection with WNV (Samuel and Diamond 2005; Keller and others 2006), DENV (Shresta and others 2004), and MVEV (Lobigs and others 2003). Enhanced infection occurred in normally resistant cell populations and tissues after flavivirus infection of $IFNAR^{-/-}$ mice, suggesting that IFN acts in part, to restrict viral tropism. The importance of type I IFN in restricting flavivirus infection has been confirmed in therapeutic disease models. Pretreatment of mice with IFN-α or inducers of IFN-α attenuates infection by SLEV, WNV, YFV, and Modoc viruses in mice and hamsters (Stephen and others 1977; Brooks and Phillpotts 1999; Leyssen and others

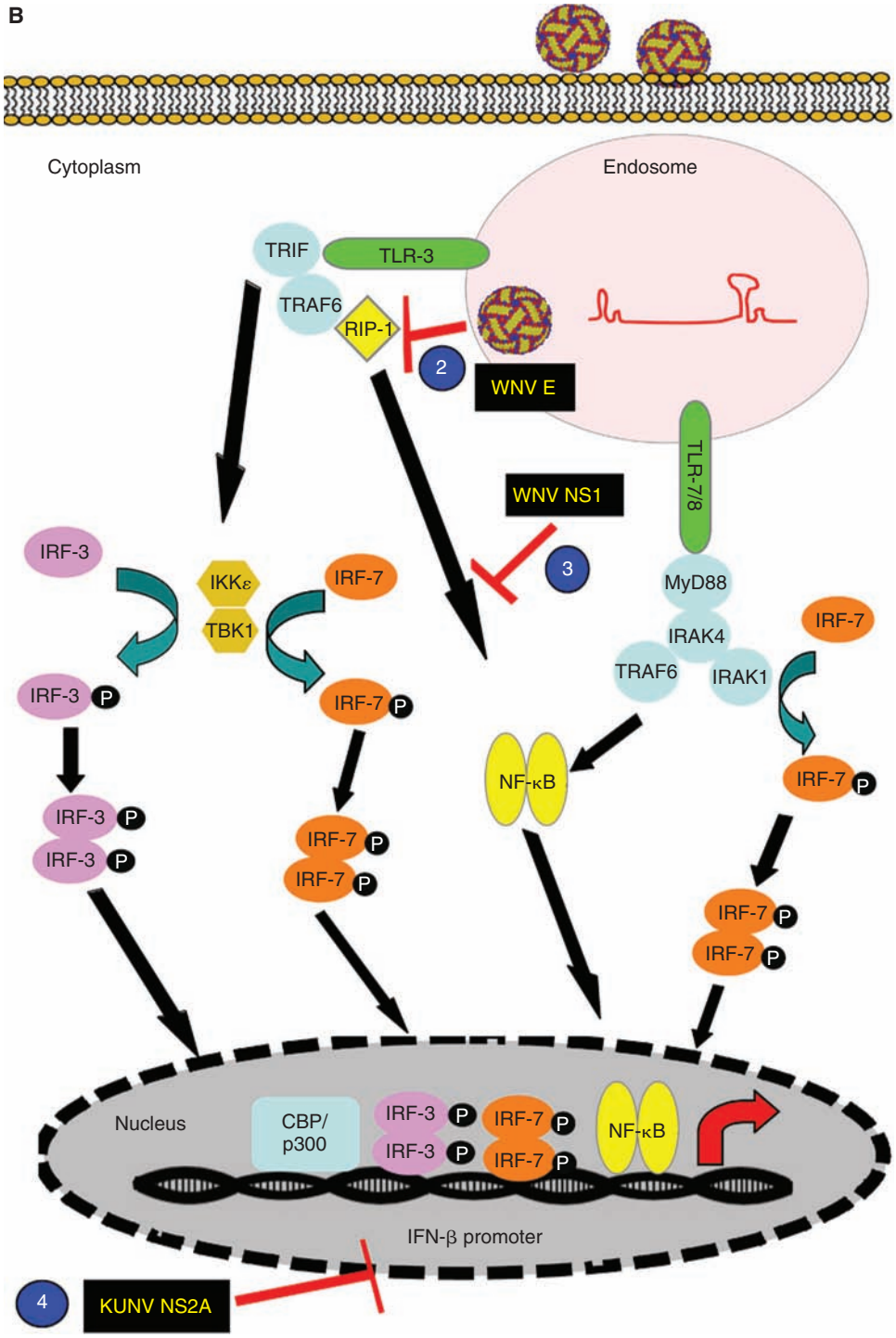

FIG. 1. (*Continued*) (**B**) Toll-like receptor (TLR) signaling cascade. In some cells, the transmembrane pathogen recognition receptors (PRRs) TLR3 and TLR7/8 in endosomes recognize dsRNA and ssRNA motifs leading to recruitment of cytoplasmic adaptor molecules (TRIF and MyD88, respectively), which initiates signaling cascades (via IKK-ε, TBK1, RIP-1, and IRAK4) that activate IRF-3, IRF-7, and NF-κB, resulting in IFN-β gene transcription. Mechanisms of evasion by flaviviruses are believed to include the following: (1) a delay in recognition of West Nile virus (WNV) RNA by RIG-I; (2) impairment of RIP-1 signaling by high mannose carbohydrates on the structural E protein; (3) attenuation of TLR3 signaling by the NS1 protein; and (4) reduction in IFN-β gene transcription by the viral NS2A protein. Cartoon is modeled after published images (Gale and Foy 2005; Best and others 2006; Keller and others 2007; Takeuchi and Akira 2007).

2001; Leyssen and others 2003; Morrey and others 2004; Julander and others 2007).

Secretion of IFN initiates a complex signal transduction cascade (Fig. 2) that results in the induction of a large number of proteins with antiviral, immunomodulatory, and cell death-promoting functions (Stark and others 1998). Binding of IFN-α and -β to their cognate common receptor (IFNAR1/IFNAR2 heterodimer) activates intracellular JAK1

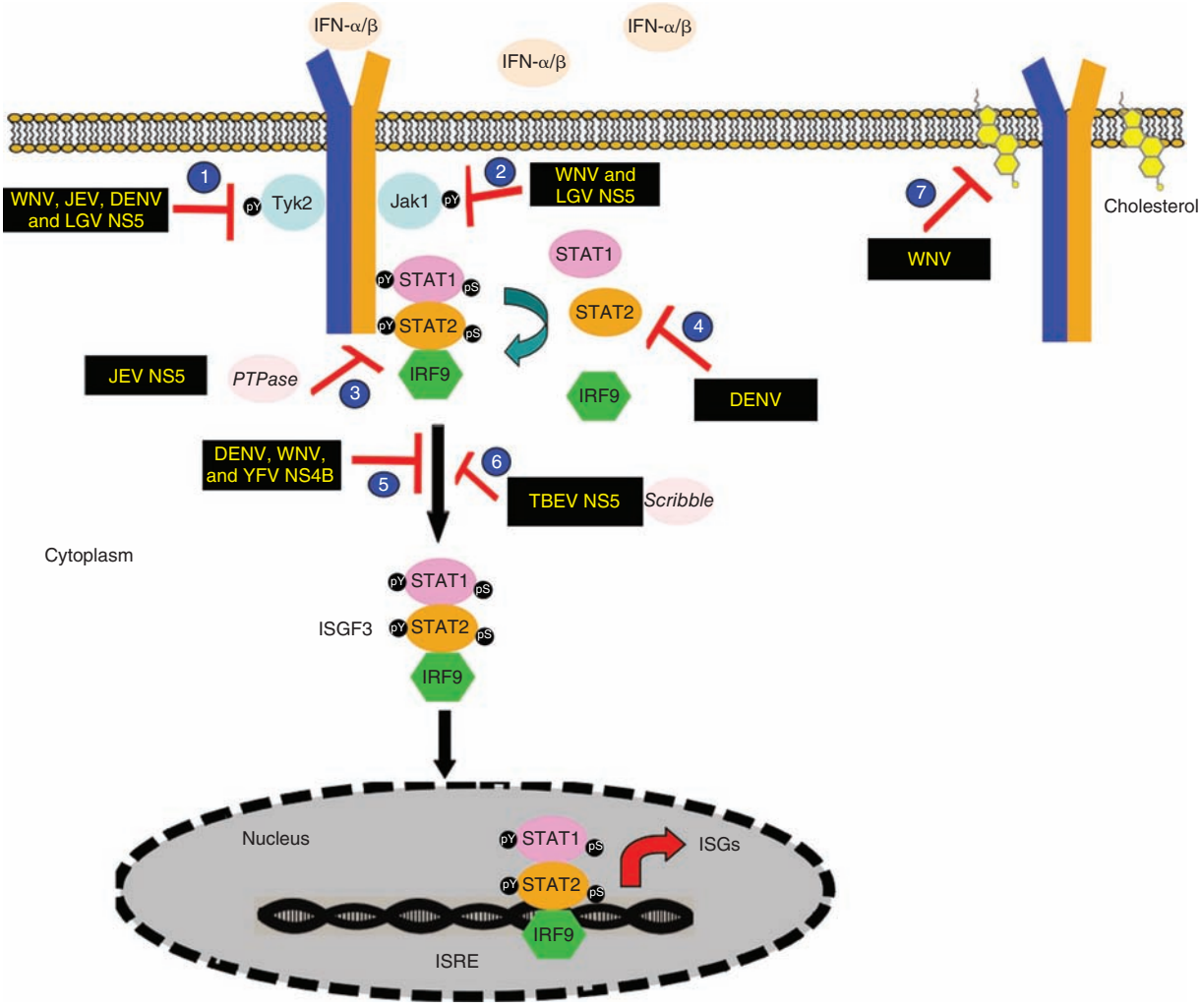

FIG. 2. Type I interferon (IFN) signaling and mechanisms of disruption by flaviviruses. Secretion of IFN by a flavivirus-infected cell results in autocrine and paracrine signaling through the heterodimeric IFN-αβ receptor (IFNAR). Binding by IFN results in activation and tyrosine phosphorylation of JAK family members (JAK1 and Tyk2) and the cytoplasmic tail of the IFN-αβR. This promotes recruitment of the STAT1 and STAT2, which themselves become phosphorylated by the JAKs. Phosphorylated STAT1 and STAT2 proteins heterodimerize, associate with IRF-9, and translocate to the nucleus, where they bind ISRE sequences to induce expression of hundreds of ISG. Mechanisms of evasion by flaviviruses are believed to include the following: blockade of phosphorylation of (1) Tyk2 and (2) JAK1 by NS5; (3) activation of a phosphotyrosine phosphatase by NS5; (4) reduction in STAT2 gene and protein expression; attenuation of STAT signaling by (5) NS4B and (6) NS5; and (7) down-regulation of the IFNAR through virus-induced redistribution of cellular cholesterol. Cartoon is modeled after published images (Gale and Foy 2005; Keller and others 2007).

and Tyk2 Janus kinases, which phosphorylate tyrosine residues on the cytoplasmic tail of the IFNAR. These phosphorylated tyrosine residues function as recruitment sites for the cytoplasmic proteins, STAT1 and STAT2, which themselves become phosphorylated by the JAKs. Phosphorylated STAT1 and STAT2 proteins heterodimerize, associate with IRF-9, and translocate to the nucleus, where they transcriptionally activate specific DNA promoter sequences to induce expression of hundreds of ISG mRNA.

Recent studies have begun to define the specific IFN-induced antiviral effector mechanisms that limit flavivirus infection. dsRNA-dependent protein kinase (PKR) and 2′-5′-oligoadenylate synthase (OAS) proteins mediate intrinsic cell resistance to WNV. PKR is activated by binding dsRNA and phosphorylates the eukaryotic translation initiation factor 2 (eIF2-α) resulting in attenuation of protein synthesis (Meurs and others 1992). RNase L is activated by 2′-5′-linked oligoadenylates that are synthesized by OAS enzymes. RNAse L functions as an endoribonuclease that cleaves viral and host RNA (Zhou and others 1993; Zhou and others 1997). RNase $L^{-/-}$ MEF and $PKR^{-/-} \times$ RNase $L^{-/-}$ macrophages supported increased WNV replication *in vitro* (Samuel and others 2006; Scherbik and others 2006). Moreover, mice deficient in both PKR and RNase L showed increased lethality following WNV infection, with higher viral loads in peripheral tissues at early time points after infection (Samuel and others 2006). The antiviral mechanism of action of PKR against WNV remains unclear: it could exert direct antiviral effects due to inhibition of viral translation, or function indirectly by inducing IFN (Gilfoy and Mason 2007). Interestingly, at least in MEF, a similar antiviral effect of PKR and RNAse L on DENV infection was not observed (Diamond and Harris 2001).

Although susceptibility to flaviviruses in mice has been mapped to a mutation in the *Oas* gene 1b, resulting in the expression of a truncated OAS isoform (Mashimo and others 2002; Perelygin and others 2002), the mechanism of control by this gene appears independent of RNAse L (Samuel and others 2006; Scherbik and others 2006) and the type I IFN-signaling pathway (Brinton and others 1982).

Antagonism of the IFN response by flaviviruses

Flaviviruses have evolved specific strategies to avoid and/or attenuate induction of IFN and its effector responses (Figs. 1 and 2). Indeed, in cell culture flaviviruses are largely resistant to the antiviral effects of IFN once infection is established (Diamond and others 2000; Anderson and Rahal 2002). This may explain in part, the relatively modest therapeutic window for IFN-α administration that has been observed clinically in animal models or humans infected with JEV, SLEV, and WNV (Brooks and Phillpotts 1999; Solomon and others 2003; Rahal and others 2004; Chan-Tack and Forrest 2005; Kalil and others 2005). Experiments by several groups have demonstrated that individual flaviviruses attenuate IFN signaling at distinct steps in the cascade.

Inhibition of IFN-β gene induction. To date, 3 independent mechanisms have been proposed by which flaviviruses minimize the induction of IFN-β.

a. IFN-β gene transcription. Studies with Kunjin (KUNV) virus, a less pathogenic lineage I WNV variant, have identified the nonstructural protein NS2A as an inhibitor of IFN-β gene transcription (Liu and others 2004; Liu and others 2006). Transgenic expression of NS2A was sufficient to suppress IFN-β transcription in Semliki Forest virus-infected cells. Incorporation of an A30P mutation of NS2A into a KUNV genome results in a virus that elicits more rapid and sustained synthesis of type I IFN; infection of this mutant virus *in vitro* and *in vivo* was highly attenuated. Nonetheless, the exact cellular target of NS2A and its mechanism of inhibition remain unknown.
b. PRR detection. Highly pathogenic WNV strains evade IRF-3-dependent recognition pathways without actively antagonizing the host defense signaling pathways (Fredericksen and Gale 2006). Indeed, WNV replication did not alter the ability of Sendai virus to activate IRF-3. Thus, virulent WNV strains appear to delay activation of PRR, such as RIG-I, through uncertain mechanisms to provide the virus with a kinetic advantage in the infected cell to elude host detection during replication at early times after infection (Keller and others 2007). In contrast, less pathogenic strains of WNV induced greater levels of IFN at early time points (Keller and others 2006).
c. TLR3-dependent responses. Activation of IRF-3 and stimulation of IFN-β transcription in response to dsRNA (poly (I:C)) are inhibited in HeLa cells infected with WNV or stably propagating a subgenomic replicon (Scholle and Mason 2005). The viral NS1 protein may mediate a part of this inhibitory effect as expression of WNV NS1 inhibited TLR3-induced transcriptional activation of the IFN-β and IL-6 transcription and NF-κB promoter activity (Wilson and others 2008). Alternatively, the high mannose carbohydrates on the viral E protein may independently block the production of IFN-β, IL-6, and TNF-α that is induced by dsRNA in macrophages. This effect was not directly dependent on TLR3 or its adaptor molecule TRIF but instead occurred downstream at the level of the signaling intermediate and NF-κB activator, receptor-interacting protein (RIP)-1 (Arjona and others 2007). Based on studies with macrophages from different age cohorts, this E protein inhibitory pathway may be dysregulated in elderly humans, leading to a pathogenic cytokine response (Kong and others 2008). Although the mechanistic basis for how specific forms of the E protein alter antiviral signaling programs remains uncertain, glycosylated E proteins can bind to and potentially signal through multiple cell surface lectins including the mannose receptor (Miller and others 2008) and CLEC5a (Chen and others 2008).

Impaired IFNAR pathway signaling. In addition to antagonizing induction of IFN-β gene responses, several flaviviruses target the JAK-STAT signaling pathway for evasion (Best and others 2006; Robertson and others 2009) to prevent the induction of antiviral ISG with possible antiviral activity. Thus, even when type I IFN is produced, it may not achieve the same inhibitory effect because of attenuated signaling capacity. As the nonstructural proteins NS2A, NS3, NS4A, NS4B, and NS5 mediate many of the viral evasion mechanisms described below, these countermeasures are largely intrinsic to infected cells. One caveat to the majority of the studies below is that the conclusions were derived from experiments in transformed cells. Even with these attenuating mechanisms, in primary macrophages and dendritic cells, flaviviruses such as WNV remain potent ISG inducers (Daffis and others 2007, 2008a, 2008b).

a. Phosphorylation of JAKs. Studies with the tick-borne Langat virus (LGV) and WNV have shown interference with phosphorylation of both JAK1 and Tyk2 (Best and others 2005; Guo and others 2005). A slight variation on this theme was observed with JEV, which showed complete inhibition of phosphorylation of Tyk2 with little effect on JAK1 phosphorylation (Lin and others 2004). Expression of a subgenomic replicon or infection of cells with DENV also inhibited Tyk2 phosphorylation and had no effect on IFNAR expression (Ho and others 2005; Jones and others 2005). However, there may be cell- or virus-specific effects as JEV also inhibits STAT1 and STAT2 activation in the setting of normal levels of Tyk2 phosphorylation (Lin and others 2008).
b. STAT gene expression. DENV has been reported to antagonize IFN function by reducing STAT2 expression (Jones and others 2005). Cell lines that stably propagated subgenomic DENV replicons were resistant to the antiviral effects of IFN-α, had reduced levels of STAT2, and blunted ISG responses. Accordingly, IFN-α but not IFN-γ responses were blocked in these cells.
c. Cholesterol redistribution. Recent studies have shown that flavivirus infection can actively promote relocalization of cholesterol to intracellular membranous sites of replication. This redistribution diminishes the formation of cholesterol-rich lipid rafts in the plasma membrane and attenuates the IFN antiviral signaling response (Mackenzie and others 2007).
d. NS proteins as specific IFN antagonists. The observation that flaviviruses antagonize IFN-signaling responses has prompted several groups to identify the viral determinants and mechanisms that mediate this process. Initial

transgenic expression studies in A549 cells with DENV showed that NS2A, NS4A, or NS4B enhanced replication of an IFN-sensitive virus by blocking nuclear localization of STAT1 (Munoz-Jordan and others 2003). Subsequent experiments showed that NS4B of DENV, WNV, and YFV partially block STAT1 activation and ISG induction (Munoz-Jordan and others 2005). Mutagenesis studies have identified a sequence determinant on WNV NS4B (E22/K24) that controls IFN resistance in cells expressing subgenomic replicons, although in cells expressing infectious virus this NS4B determinant did not regulate the IFN response, suggesting an independent role for structural genes (Evans and Seeger 2007).

NS5 has been reported as the primary nonstructural protein responsible for attenuating JAK-STAT signaling after LGV, JEV, and TBEV infection (Best and others 2005; Lin and others 2006; Werme and others 2008). However, the mechanism of NS5 inhibition may have virus-specific characteristics. For TBEV, a sequence in the methyltransferase domain of NS5 binds the PDZ protein scribble to inhibit JAK-STAT signaling (Werme and others 2008). For JEV, the N-terminal 83 residues of NS5 inhibit JAK-STAT signaling through a protein-tyrosine phosphatase-dependent mechanism (Lin and others 2006). Finally, for LGV, the JAK-STAT inhibitory domain was mapped to sites within the RNA-dependent RNA polymerase domain (Park and others 2007).

Impaired IFN effector functions. Although flaviviruses devote a significant segment of their genome to inhibiting JAK-STAT signaling, they may also target individual downstream antiviral effector molecules. Viperin is a candidate antiviral ISG with inhibitory activity against hepatitis C, influenza, HIV, and Sindbis viruses (Rivieccio and others 2006; Wang and others 2007; Zhang and others 2007; Jiang and others 2008), possibly because of its ability to alter lipid raft formation (Wang and others 2007). JEV, however, counteracts the antiviral activity of viperin by promoting rapid proteasome-dependent degradation (Chan and others 2008). The mechanism of this inhibition remains unclear as transfection of individual JEV proteins failed to recapitulate the phenotype suggesting a combined effect of viral proteins or replication is required.

Summary

The use of animal and cell culture models has fostered an improved understanding of the balance between flavivirus pathogenesis and immune control. IFN responses limit infection flaviviruses and not surprisingly, as a group, these successful mammalian pathogens have developed countermeasures to facilitate infectivity and transmission. In the last 5 years, the field has learned the identity of specific PRR that detect entry and infection by flaviviruses and initiate a protective IFN response, and which viral proteins allow evasion of the response. The next decade will likely provide us with insight into mechanisms as several key questions remain unanswered. These include (a) identification of the specific PAMP on flaviviruses that are recognized by PRR. Experiments with hepatitis C virus have identified homopolyuridine and homopolyriboadenine motifs as substrates for RIG-I (Saito and others 2008). What additional recognition motifs will there be for flaviviruses, which lack these sequences and yet, are still recognized RIG-I?; (b) What are the particular ISG that mediate antiviral effector functions against flaviviruses? Although some molecules (eg, PKR and RNAse L) have been identified, they do not account for the majority of inhibitory activity that is generated after exogenous IFN treatment or endogenous IFN induction in most cells? What ISG have key inhibitory functions against flaviviruses? Will IFN-induced microRNA that regulate transcription of host genes essential for viral replication explain some of the inhibitory effect (Pedersen and others 2007; Mahajan and others 2009)?; (c) How do flavivirus proteins disable the cellular IFN induction and signaling response? What are the precise host target proteins and the molecular and structural basis of the antagonism?; and (d) Are virulence determinants that antagonize specific IFN induction or effector pathways a quality that defines highly virulent and disease causing flavivirus strains? As these basic mechanisms are explored and characterized, the field undoubtedly will gain insight into fundamental cellular responses as well mechanisms of viral pathogenesis. It is this information that may facilitate the design of novel vaccine or targeted therapeutic strategies and enhance our understanding of how pathogens of all types cause disease.

Acknowledgments

M.S.D. would like to thank T. Pierson, S. Daffis, and K. Szretter for critical comments and suggestions on the manuscript and M. Gale for stimulating discussions.

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Received 29 June 2009/Accepted 29 June 2009