REVIEW

Innate immune response to La Crosse virus infection

Katherine G. Taylor · Karin E. Peterson

Abstract Viral encephalitis represents a significant, and costly, public health threat particularly for high-risk pediatric populations. An emerging mosquito-borne pathogen endemic to the United States, La Crosse virus (LACV) is one of the most common causes of viral encephalitis in children in the United States. However, no licensed therapeutics or vaccines currently exist for treatment. Hampering development efforts, the host response to LACV and its role in disease pathogenesis has only recently been examined. In this review, we discuss the current understanding of innate immune response in the context of viral pathogenesis and host susceptibility to LACV. In addition, we address the need for a clearer understanding of the early host–virus interactions in LACV infections as it relates to viral pathogenesis in the central nervous system.

Keywords La Crosse · Encephalitis · Innate · SARM1 · Interferon

Introduction

La Crosse virus (LACV) is a member of the genus *Orthobunyavirus*, family Bunyaviridae that circulates throughout the eastern and mid-western United States. LACV typically causes a mild, febrile disease in the majority of infections; however, a subset of pediatric patients develops acute meningeoencephalitis. LACV represents the primary cause of viral encephalitis in children in the United States with reported rates ranging from 5 to 8 % of acute central nervous system (CNS) disease (Balfour Jr. et al. 1973; Haddow et al. 2009; Haddow and Odoi 2009). Incidence

of CNS manifestations is highest in children under the age of 15, and severe disease is associated with long-lasting neurological sequelae. Adult infections may be limited to the periphery by an earlier, more robust anti-viral immune response. However, despite the well-established age-based susceptibility for LACV and other encephalitic viruses, the means of preventing pediatric susceptibility to viral invasion of the CNS is limited by poor mechanistic understanding of age-related viral pathogenesis/protection.

While annual incidence of human disease is between 20 and 30 cases per hundred thousand (Balfour Jr. et al. 1973; Haddow et al. 2009; Haddow and Odoi 2009), the mild nature of the majority of infections combined with the high prevalence of LACV antibodies in human populations indicates that infections are underreported (Grimstad et al. 1984; Szumlas et al. 1996a). Interestingly, analysis of HLA diversity in patients with acute, LACV encephalitis indicates an immunogenetic component may contribute to susceptibility to encephalitis (Case et al. 1993). However, other factors including exposure to vector, dose of virus, and age of the host also influence incidence, morbidity, and mortality (Calisher 1994).

Viral genome and structure

LACV has a trisegmented negative strand RNA genome which encodes small, middle, and large segments responsible for production of the structural and non-structural proteins (Schmaljohn and Nichol 2007). The L segment encodes the RNA-dependent RNA-polymerase necessary for viral RNA replication and mRNA synthesis. The M segment encodes the viral glycoproteins, G1 and G2. The two glycoproteins enable viral attachment and entry into the host cell and are considered important determinants of virulence (Gonzalez-Scarano et al. 1988; Gonzalez-Scarano et al. 1985). The S segment encodes the nucleocapsid protein (N) and the non-structural protein

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(NSs). NSs inhibits the host anti-viral response (Gonzalez-Scarano et al. 1988; Gonzalez-Scarano et al. 1985). The genome of LACV is genetically stable; however, specific amino acid differences in RNA polymerase have been associated with human isolates and may influence viral pathogenesis in humans (Bennett et al. 2007).

Viral transmission and dissemination

The widening geographic range of host and vector combined with the severe clinical symptoms in pediatric populations make LACV an important emerging infectious disease in the United States (Lambert et al. 2010; Leisnham and Juliano 2012). LACV circulates between the primary mosquito vector *Aedes triseriatus* and several small mammal species (Scheidler et al. 2006; Szumlas et al. 1996b; Watts et al. 1974). Recently, other mosquito vectors including *Aedes albopictus* and *Aedes japonicus* have been identified indicating potential for an expanded geographic range for LACV infections (Jones et al. 1999; Lambert et al. 2010; Leisnham and Juliano 2012; Reese et al. 2010).

Humans become infected following the bite of an infected mosquito. The two viral glycoproteins mediate host cell attachment and the initial steps of viral replication. In mammalian cells, LACV enters via clathrin-mediated endocytosis and Rab-5-mediated trafficking to early endosomes (Hollidge et al. 2012). Dissemination of virus to the CNS is hypothesized to occur following robust replication in peripheral tissue leading to serum viremia that transmits virus to the brain (Griot et al. 1994; Janssen et al. 1984a). Although the route of LACV entry to the CNS is not clearly defined, one entry mechanism may be the direct infection of the olfactory epithelium (Bennett et al. 2008).

Most of the current understanding of transmission, dissemination, and pathogenesis of LACV in mammals has been derived from murine models, although a few studies have been completed in non-human primates (Bennett et al. 2008; Bennett et al. 2011). Suckling and weanling mice develop encephalitis via multiple routes of infection including intraperitoneal (ip), subcutaneous (sc), intranasal (in), or intracerebral (ic) inoculation (Bennett et al. 2008; Hammon et al. 1952; Janssen et al. 1984a; Johnson and Johnson, 1968; Johnson 1983). However, mice older than 30 days are generally asymptomatic following peripheral routes of inoculation mimicking age-related resistance seen in human populations (Table 1). Interestingly, direct ic inoculation in older mice results in similar clinical disease to that seen in suckling mice (Bennett et al. 2008; Johnson 1983). These models suggest that limiting peripheral virus replication and/or access to the CNS are key steps preventing LACV neurovirulence in adults.

Generation and regulation of the type I IFN response to LACV infection

The ability of the host to initiate an anti-viral environment is a critical step in the prevention of viral pathogenesis. One of the key antiviral events during LACV infection is the production of type I interferons (IFN) including IFNα and IFNβ. Both adult and weanling mice deficient in the type I IFN receptor 1 (*Ifnar1*) are more susceptible to LACV-induced neurological disease than wild-type mice (Table 2) (Hefti et al. 1999; Operschall et al. 1999; Pavlovic et al. 2000). Furthermore, our recent studies demonstrate that deficiency in the signal transduction molecules, interferon response factors (IRF) 3 and 7, also leads to earlier onset of LACV-induced neurological disease (Mukherjee et al. 2013). IRF3 and IRF7 are activated following stimulation of pattern recognition receptors (PRRs), membrane bound or cytoplasmic cellular receptors that recognize foreign particles such as viral RNA, viral DNA, or bacterial components. Studies in HEK cells and in cultured neurons demonstrate that the cytoplasmic RNA-helicase PRR, RIG-I, is induced following LACV infection (Mukherjee et al. 2013; Verbruggen et al. 2011). RIG-I binds to mitochondria anti-viral signaling protein (MAVS) which then leads to the activation of IRF3, IRF7, and NFκB, resulting in the production of type I IFN and proinflammatory cytokine responses.

The type I IFN response is the best-characterized cytokine response to LACV infection and has a direct anti-viral effect reducing viral load and preventing neurological disease. Early studies on LACV indicated that virus replication was inhibited by stimulation of cells with poly I:C, a molecule that activates PRR and leads to type I IFN production, or by direct addition of IFN in primary human fetal glial cell cultures (Luby 1975). IFN can induce anti-viral activity through production of MxA, a GTPase with antiviral activity against RNA viruses. MxA is a dynamin-like protein that has multiple cellular functions including maintenance of cellular homeostasis. MxA in cell culture reduced LACV viral titers preventing accumulation of both viral transcripts and proteins indicating an early mechanism of action (Frese et al. 1996; Miura et al. 2001). In vivo, MxA confers resistance to *Ifnar1*$^{-/-}$ mice to LACV infection and restricts LACV entry to the central nervous system (Hefti et al. 1999).

MxA acts to reduce viral replication by binding and relocating the nucleocapsid protein of LACV into membrane-associated perinuclear complexes in a subcompartment of the smooth endoplasmic reticulum. These MxA–nucleocapsid complexes are found in conjunction with both caspase recruitment domain-containing protein 16 (COP-I) and vesicular tubular membranes in the smooth endoplasmic reticulum (Reichelt et al. 2004). zutation of MxA at the carboxy terminus inhibits binding to the nucleocapsid protein, resulting decreased antiviral activity against LACV (Kochs et al. 2002). Thus, a direct anti-viral effect

Table 1 Age-related resistance to LACV is dependent on route of infection

Route	Strain	Virus (source)	Dose[a]	Neonates[b]	Weanling[c]	Weaned[d]	Reference
i.c.	BALB/c	LACV, Thompson, 1965	1E−01		100 %	50 %	Janssen et al. 1984a, b
	CD-1	LACV, Thompson, 1965	7E+02	**100 %**			Janssen et al. 1984a, b
	Outbred	LACV, 192 strain	1E+03	**100 %**		100 %	Johnson and Johnson 1968
i.n.	Swiss Webster	LACV/human/1960	1E+01		0 %		Bennett et al. 2008
	Swiss Webster	LACV/human/1960	1E+03		100 %		Bennett et al. 2008
i.p.	CD-1	LACV, Thompson, 1965	1E−01	**50 %**			Janssen et al. 1984b
	Swiss Webster	LACV/human/1960	1E+01		~20 %		Bennett et al. 2008
	BALB/c	LACV, Thompson, 1965	1E+03		50 %		Janssen et al. 1984b
	C57BL/6	LACV/human/1978	1E+03		100 %		Mukherjee et al. 2013
	Outbred	LACV, 192 strain	*1E+03*	*100 %*	20 %	0 %	Johnson and Johnson 1968
	Swiss Webster	LACV/human/1960	1E+03		100 %		Bennett, 2008
	129sv	LACV, Thompson, 1972	*1E+05*			0 %	Pavlovic, 2000
	BALB/c	LACV, Thompson, 1965	1E+05			50 %	Janssen et al. 1984a, 1984b
	CD-1	LACV, Thompson, 1965	1E+05		100 %	0 %	Pekosz et al. 1995
s.c.	CD-1	LACV, Thompson, 1965	7E+02	**100 %**			Janssen et al. 1984a, 1984b
	Outbred	LACV, 192 strain	*1E+03*	**100 %**		0 %	Johnson and Johnson 1968

[a] PFU/mouse, TCID50 per mouse in italics

[b] Twenty-four hours to 8 days of age, less than 3 days of age in bold, 8 days of age in bold italics

[c] Three to 4 weeks of age

[d] Four weeks or older

for IFN-induced MxA is a key component of the host anti-viral response.

Due to the importance of type I IFNs in regulating virus replication and spread in the host, arboviruses universally encode non-structural proteins that interfere with the type I IFN response. During LACV infection, the NSs protein indirectly antagonizes type I IFN signaling (Blakqori et al. 2007; Verbruggen et al. 2011). LACV inhibits IFN induction in mouse fibroblasts; however, LACV mutants lacking NSs did not (Blakqori et al. 2007). LACV containing a NSs mutation is highly attenuated in wild-type mice, but not in *Ifnar1*^{−/−} mice indicating that the NSs has an important role

Table 2 Immune deficiency alters LACV pathogenesis

Deficiency	Strain	Route	Virus (source)	Dose[a]	Age	% clinical	MTD[b]	Reference
wildtype	C57BL/6	ip	LACV/human/1978	1E+03	3 weeks	100 %	6	Mukherjee et al. 2013
Irf3−/−,Irf7−/−	C57BL/6	ip	LACV/human/1978	1E+03	3 weeks	100 %	4	Mukherjee et al. 2013
Myd88−/−	C57BL/6	ip	LACV/human/1978	1E+03	3 weeks	93 %	8	Mukherjee et al. 2013
Mavs−/−	C57BL/6.129	ip	LACV/human/1978	1E+03	3 weeks	90 %	6	Mukherjee et al. 2013
Sarm1−/−	C57BL/6	ip	LACV/human/1978	1E+03	3 weeks	60 %	9	Mukherjee et al. 2013
wildtype	C57BL/6	ip	rLACV	1E+04	3 weeks	80 %	**6**	Blakqori et al. 2007
Ifnar1−/−	C57BL/6	ip	rLACV	1E+04	3 weeks	100 %	**4**	Blakqori et al. 2007
Ifnb1−/−	C57BL/6	ip	rLACV	1E+04	3 weeks	80 %	**5**	Blakqori et al. 2007
Ifnar1−/−	C57BL/6x SJL	ip	LACV, Thompson et al. 1965	*1E+05*	6 weeks	94 %	7	Hefti et al. 1999
Mxa+/+, Ifnar1 −/−	C57BL/6x SJL	ip	LACV, Thompson et al. 1965	*1E+05*	6 weeks	44 %	8	Hefti et al. 1999
wildtype	129Sv	ip	LACV, Thompson, 1972	*1E+05*	6–8 weeks	0 %	–	Pavlovic et al. 2000
Ifnar1−/−	129Sv	ip	LACV, Thompson, 1972	*1E+05*	6–8 weeks	100 %	**8**	Pavlovic et al. 2000
Ifnar1−/−	129Sv	ip	LACV, Thompson, 1972	*1E+05*	6–8 weeks	100 %	**10**	Schuh et al. 1999

[a] PFU per mouse, TCID50 per mouse in italics

[b] Mean time to disease, extrapolated from graph in bold

in controlling IFN response in vivo (Blakqori et al. 2007). NSs acts by degrading transcription of RNA polymerase II subunit RBP1. RBP1 is induced following RIG-I recognition of the virus, causing host cell transcriptional shutdown and prevention of type I IFN production (Verbruggen et al. 2011). Thus, virus replication and spread in the host can be regulated both by the host's ability to mount an effective type I IFN response to LACV infection as well as by the ability of LACV NSs protein to limit the generation of that type I IFN response.

Protective and pathogenic contributions of the innate immune response to LACV in the CNS

Once the virus reaches the CNS, the precise role of type I IFN is less well understood. In the CNS, LACV primarily infects neurons ultimately causing cell death associated with neuronal degeneration, apoptosis, and necrosis (Balfour Jr. et al. 1973; Janssen et al. 1984a; Johnson and Johnson, 1968; Johnson 1983; Jortner et al. 1971; Kalfayan 1983; Pekosz and Gonzalez-Scarano 1996; Pekosz et al. 1996; Thompson and Evans 1965; Thompson et al. 1965). Neurons are equally susceptible to infection regardless of age of the host though distribution of viral antigen and lesions differs in the brain (Hammon and Reeves 1952; Hammon et al. 1952; Janssen et al. 1984b; Johnson and Johnson, 1968; Johnson 1983). CNS glia do not appear to be susceptible to LACV infection. Type I IFN expression correlates with the kinetics and distribution of viral antigen (Delhaye et al. 2006; Lienenklaus et al. 2009). A subset of neurons, macrophages, and ependymal cells can produce type I IFN following LACV infection and recently both astrocytes and microglia were identified as IFN-β-producing cell types in the CNS of LACV-infected mice (Delhaye et al. 2006; Kallfass et al. 2012). In addition, both neurons and other CNS cells can respond to type I IFN (Delhaye et al. 2006). However, our studies with cultured cortical neurons from $Ifnar1^{-/-}$ or $Irf3^{-/-}$ $Irf7^{-/-}$ mice indicates that neurons underwent LACV-induced cell death at a similar rate to wild-type neurons suggesting that the type I IFN response may not have an autocrine protective effect (Mukherjee et al. 2013).

Instead of being protective, our recent studies indicate that the activation of the RIG-I and MAVS signaling pathway in neurons contributes to neuronal apoptosis during LACV infection (Fig. 1). Activation of RIG-I leads to the activation of MAVS at the mitochondria and mitochondrial-associated membranes. The subsequent signaling pathway in LACV-infected neurons leads not only to the production of type I IFN, but also induces the upregulation of the adaptor molecule SARM1 (Mukherjee et al. 2013). SARM1 is normally expressed in the cell bodies of neurons and has an active role in dendrite formation (Chen et al. 2011). However, increased expression of SARM1 in neurons, during virus infection, axonal damage, or oxygen glucose depravation, can result in cell death (Kim et al. 2007; Mukherjee et al. 2013; Osterloh et al. 2012). Neurons from $Sarm1^{-/-}$-deficient mice or treated with $Sarm1$ siRNA have reduced incidence of cell death following LACV infection compared to neurons from wild-type controls, despite similar amounts of viral RNA (Mukherjee et al. 2013). Similar results were also observed in vivo, where $Sarm1^{-/-}$-deficient mice developed clinical disease at a reduced rate compared to wild-type controls (Table 2) (Mukherjee et al. 2013).

We found that SARM1-mediated cell death during LACV infection is associated with mitochondrial localization of SARM1, production of reactive oxygen species, mitochondrial damage, and apoptosis. Deficiency in SARM1 inhibited the oxidative stress response to LACV infection both in vitro and in vivo (Mukherjee et al. 2013). A mitochondrial mediated mechanism of neuronal death by LACV correlates with the finding that human BCL-2 expression, which inhibits mitochondrial pathway of apoptosis, reduces the cytopathic effects of LACV infection in cell culture systems (Pekosz et al. 1996).

Other components of the innate immune response may also influence viral pathogenesis following viral infection of the CNS. A proinflammatory cytokine response is induced following infection with LACV in both the periphery and the brain with production of IL-6 and IL-12p40 in serum and these in addition to IL1α, IL-1β, and several chemokines in the brain (unpublished observations). Similar cytokine profiles have been demonstrated for other encephalitic viruses and associated with viral pathogenesis particularly in the CNS (Cho and Diamond 2012; Gray et al. 2012; Taylor et al. 2012). Administration of IL-12 or GM-CSF encoded by a plasmid prior to LACV infection $Ifnar1^{-/-}$ mice increases survival rates suggesting a protective role (Operschall et al. 1999; Pavlovic et al. 2000). However, production of these factors may also contribute indirectly to CNS infection. For example, proinflammatory cytokine expression may impact permeability of the blood–brain barrier (BBB) and contribute to LACV infection of the CNS. Compromising the BBB may directly influence pathogenesis by allow entry of virus or virus-infected cells into the brain. Additionally, alterations to the BBB may influence the recruitment of inflammatory cells to the CNS.

The types and mechanism of action of the cellular infiltrates in the brain may also be key in understanding the pathogenesis of La Crosse virus infection of the CNS. Histological analysis of tissues from both mouse and humans show leukocyte infiltration and perivascular cuffing of lymphocytes following LACV infection (Bennett et al. 2008; Kalfayan 1983; Thompson et al. 1965). This includes focal aggregates of macrophages and neutrophils as well as perivascular cuffing of CD3+ lymphocytes (Bennett et al.
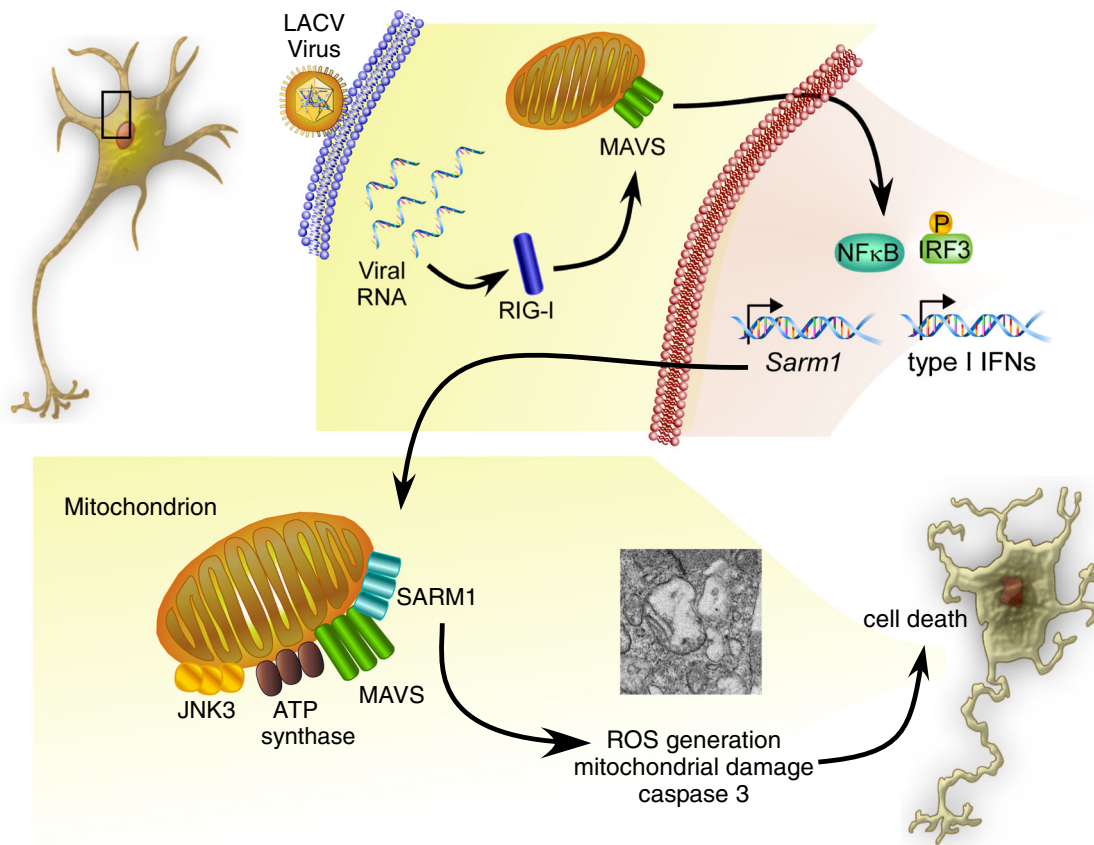
Fig. 1 Bunyavirus infection activates RIG-I signaling pathways in neurons. RIG-I activation leads to induction of the MAVS signaling pathway MAVS signaling leads to upregulation of type I IFN and SARM1 in neurons SARM1 localizes with MAVS at the mitochondria inducing an oxidative stress response leading to neuronal apoptosis

2008; Thompson et al. 1965). The role of these cells in LACV pathogenesis is not clear, although it may be similar to other neurovirulent viruses. For example, in flavivirus infection, both CD4+ T-cells and CD8+ T cells can control virus infection and mediated viral clearance from the CNS (Shrestha and Diamond 2004; Sitati and Diamond 2006). In contrast, natural killer cells contribute to pathogenesis in a murine model of alphavirus infection (Taylor et al. 2012). Examining the recruitment of inflammatory cells into the CNS and the effect of these cells on LACV replication and neuronal damage in the brain and spinal cord will provide a better understanding of how the innate immune response influences viral pathogenesis.

Conclusion

The innate immune system supports both pathogenic and protective mechanisms following infection and, as such, represents a paradigm for the host response to viral infection. Complicating the functional picture of the innate immune response, the same response may elicit different tissue-dependent effects. For instance, peripheral stimulation of

anti-viral recognition pathways leads to production of type I IFN responses which inhibit viral replication, control LACV infection and possibly affect the ability of virus to infect the CNS (Blakqori et al. 2007; Hefti et al. 1999; Pavlovic et al. 2000; Schuh et al. 1999). However, activation of the same signaling pathway in the context of the CNS contributes to neuronal cell death (Mukherjee et al. 2013). Thus, activation of the same pathway in the periphery and CNS results in protection or pathogenesis respectively, and exemplifies the dual nature of the immune system in the CNS.

Understanding the contribution of the type I IFN response to disease in specific tissues is critical and may explain key epidemiological features of LACV infection. The protective, peripheral type I IFN response may contribute to age-related resistance. For instance, adult $Ifnar1^{-/-}$ mice are highly susceptible to peripheral infection, and adult wild-type mice are not (Table 2). Thus, age-related resistance to LACV may depend on a strong peripheral type I IFN response. The cells in the periphery producing type I IFN, the signaling molecules and receptors inducing type I IFN signaling, and the subsequent downstream effects have not been explored. Furthermore, while NSs inhibits type I IFN, the more global effects of LACV infection in vivo on the type I IFN system

are poorly understood (Blakqori et al. 2007). Examining the peripheral innate immune response between susceptible, young mice and resistant, older mice may result in a greater understanding of why LACV-induced neurological disease is age restricted.

In contrast, the innate immune response in the CNS appears to be a contributing factor to the disease process. In the CNS, as opposed to the periphery, the activation of the innate immune response leads to neuronal apoptosis through MAVS-induced expression of SARM1(Mukherjee et al. 2013). Thus, the means or ability of the innate immune response in the CNS to protect against neuronal damage requires further study. Understanding this mechanism of innate immune-induced autonomous neuronal damage should provide insight into potential therapeutic targets to limit neuronal damage during virus infection. Furthermore, determining if SARM1-mediated cell death is only limited to infected neurons or whether activation of the innate immune response also induces death pathways in uninfected neurons through activation of other PRRs will be important. Several studies have shown that TLR stimulation of neurons can induce apoptosis in the absence of infection (Lehnardt et al. 2003; Ma et al. 2006). This suggests that a proinflammatory environment may lead to the generation of damage-associated molecular patterns that contribute to the disease process.

Some of the influence of the innate immune response on LACV pathogenesis may be inferred from other encephalitic viruses; however, distinctions between different encephalitic viruses limit our ability to predict the influence of a particular pathway to disease development. The ability of viruses to manipulate the innate immune system through viral proteins may partially explain the divergent nature of responses. Viral proteins often target the type I interferon response, but they inhibit this response using different mechanisms that may alter the overall host inflammatory response to virus infection. Examining the influence of the host innate immune response to different encephalitic viruses in the CNS will allow us to develop a better understanding of inflammation in the brain. While important in understanding the pathogenesis of individual viral infections, defining the innate immune responses in the CNS will also provide a broader sense of the ability of cells of the CNS to react to danger and damage signals as well as the triggers that lead to specific protective or damaging pathways.

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