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INNATE IMMUNE CONTROL OF ALPHAVIRUS INFECTION

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Abstract

Alphaviruses are important human pathogens that cause diseases ranging from acute and chronic polyarthralgia to encephalitis. Transmitted by mosquito vectors, alphaviruses have high potential for emergence and have initiated several recent epidemics. The innate immune response is critical for controlling the acute phase of alphavirus disease, and the induction of type I interferon (IFN) is essential in this response. In this review, we discuss our current understanding of innate host sensors that initiate antiviral responses following alphavirus infection, and the IFN-induced effector proteins that limit alphavirus replication and dissemination.

INTRODUCTION

The genus Alphavirus (family *Togaviridae*) includes ~30 currently recognized viral species [1]. These single-stranded, positive-sense RNA viruses are primarily arboviruses transmitted between mosquito vectors and vertebrate hosts. Alphaviruses include human pathogens that, in some cases, have recently caused explosive epidemics involving millions of people [2,3]. Although infections with alphaviruses such as chikungunya (CHIKV), o'nyong nyong (ONNV), Ross River (RRV), Mayaro (MAYV), and Sindbis (SINV) viruses are typically not life threatening, they can cause severe and often chronic myalgia and arthralgia. In addition, CHIKV is associated with severe neurological disease in the young and the elderly [4]. Other alphaviruses, including Venezuelan (VEEV), western (WEEV), and eastern (EEEV) equine encephalitis viruses can cause encephalitic and sometimes fatal infections in humans [5]. Thus, there is a pressing need to improve an understanding of the innate immune mechanisms that control alphavirus infection.

Type I IFN is essential for control of alphavirus infection

The innate immune response provides a first line of defense against alphavirus infection. Central to this response is type I interferon (IFN), as mice deficient in the IFN receptor (*Ifnar1*^{-/-}) are highly susceptible to infection with numerous alphaviruses [6–11].

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Furthermore, attenuated strains of alphaviruses often display increased virulence in *Ifnar1*^{-/-} mice [8,12–14]. Experiments in bone marrow chimeric mice revealed that IFN signaling in nonhematopoietic cells is essential for CHIKV control, while IFN signaling in hematopoietic cells is dispensable [15]. Nevertheless, both cell types likely contribute to IFN production, as expression of transcription factors responsible for IFN induction (IRF3/7) by either hematopoietic or nonhematopoietic cells is sufficient for CHIKV control [16]. However, our understanding of the specific cell types that produce IFN in response to alphavirus infection and the pathogen recognition receptors (PRRs) that induce this response is limited.

Innate Sensing of Alphaviruses

Viral RNAs serve as pathogen associated molecular patterns (PAMPs) that are recognized by host PRRs, including Toll-like receptors 3, 7, and 8 (TLR3, TLR7, and TLR8), and RIG-I-like receptors (RLRs) [17]. Engagement of PRRs activates signaling cascades, which ultimately induce IFN production. While IFN is essential for alphavirus control, the relative contributions of these PRRs during alphavirus infection have not been fully elucidated. The host also can respond to viruses through additional receptors, including Nod-like (NLRs) and C-type lectin (CLRs) receptors [18,19]. The role of NLRs and CLRs in alphavirus infection has recently begun to be appreciated (Figure 1).

TLRs

TLRs are a family of conserved pathogen recognition receptors, and all but TLR3 signal through the adaptor protein MyD88 [20]. Studies in *MyD88*^{-/-} mice suggested that TLR signaling may contribute to control of CHIKV infection, as *MyD88*^{-/-} mice had increased viremia and enhanced dissemination compared with WT mice [15,21]. Similarly, RRV-infected *MyD88*^{-/-} mice exhibited more severe disease and had increased viral tissue burdens [22]. These effects were likely due to sensing of RRV by TLR7, as *MyD88*^{-/-} and *Tlr7*^{-/-} mice had similar viral tissue burdens and disease progression following RRV infection [22].

TLR3 appears to restrict CHIKV, although there are conflicting reports in the literature. One study found that *Tlr3*^{-/-} mice were more susceptible to CHIKV infection, as evidenced by increased viremia and tissue burdens [23]. Moreover, CHIKV infected *Tlr3*^{-/-} mice had exacerbated inflammation in the inoculated foot that was accompanied by a massive infiltration of myeloid cells compared with CHIKV-infected WT mice. Bone marrow chimeric mice revealed that TLR3 expressed by hematopoietic cells contributes to control of CHIKV viremia, whereas expression of TLR3 by nonhematopoietic cells enhanced inflammation [23]. In contrast, a separate study found no difference in viral tissue burdens in CHIKV-infected wild type (WT) or *Tlr3*^{-/-} mice [15]. Regardless, the protective role of TLR3 during CHIKV infection is further supported by studies in mice lacking TRIF, an adaptor protein downstream of TLR3, as *Trif*^{-/-} mice infected with CHIKV had increased viremia and swelling in the inoculated foot [21].

Whether TLR sensing is critical for control of other alphavirus infections has not been thoroughly investigated. However, a neuroadapted strain of SINV was equally virulent in

WT, *Tlr3*^{-/-}, and *MyD88*^{-/-} mice, with no observed difference in IFN production within the CNS [24]. A second study found that *MyD88*^{-/-} and WT mice were equally susceptible to the AR86 strain of SINV, while *Trif*^{-/-} mice succumbed to infection only slightly more rapidly than WT mice [25]. Thus, findings with one alphavirus species may not be broadly applicable to all alphaviruses.

RLRs

RLRs, including RIG-I and MDA5, are a family of cytoplasmic RNA helicases that signal through the adaptor molecule MAVS to activate transcription factors IRF3 and IRF7, driving transcription of IFN and other proinflammatory cytokines [17]. Studies in cell culture demonstrated MAVS is essential for the IFN response to CHIKV, VEEV, and SINV [15,26,27]. RLRs also restrict CHIKV infection *in vivo*, as *Mavs*^{-/-} mice had increased viremia and increased swelling in the inoculated foot [15,21]. Moreover, serum IFN levels were reduced during CHIKV infection of *Mavs*^{-/-} mice [21]. *Mavs*^{-/-} mice also were more susceptible to SINV, with decreased mean survival times and increased viral loads in the brain and spinal cord relative to WT mice [25]. Both RIG-I and MDA5 contribute to IFN induction in response to alphavirus infection *in vitro* [15,27]. Work *in vivo* also suggests RIG-I and MDA5 are redundant, as CHIKV tissue burdens in mice individually deficient for either *RigI* or *mda5* were not remarkably different from WT mice [15]. Experiments using bone marrow chimeric mice revealed that nonhematopoietic cell expression of MAVS is critical for control of CHIKV infection [16]. This is in contrast to the requirement of hematopoietic cell expression of TLR3 for efficient control [23], suggesting that distinct viral sensors play unique roles in different cell types.

NLRs

NLRs respond to a variety of PAMPs and are a crucial component of the innate immune response [28]. Some NLRs induce inflammasome formation, which activates caspase-1 and the release of proinflammatory cytokines, including IL-1 β . IL-1 β is up-regulated by multiple alphaviruses [29–32]. Moreover, IL-1 β deficient mice are more resistant to SINV [29], and IL-1 β elevation serves as a biomarker of CHIKV disease severity in patients [33]. Despite this, a role for NLRs in alphavirus replication and pathogenesis has only recently been established. CHIKV induced inflammasome activation *in vitro*, and knockdown of caspase-1 in fibroblasts moderately increased CHIKV replication [34]. Additionally, NLRP3 was recently reported to respond to alphavirus infection *in vivo*. Treatment of mice with NLRP3 or caspase-1 inhibitors led to less severe disease in CHIKV- and RRV-infected mice, including reduced osteoclastogenic bone loss [35]. Other NLRs (NLRP1, NLRP6 and NLRC4) were transcriptionally up-regulated in the ankle joints of mice following CHIKV infection [35], suggesting additional NLRs also may respond to alphaviruses. More work is necessary to fully elucidate the mechanisms by which NLRs contribute to alphavirus pathogenesis.

CLRs

CLRs recognize viral glycans and can activate antiviral responses, including phagocytosis and antigen presentation [36]. However, some alphaviruses exploit CLRs to access host cells. For example, DC-SIGN and L-SIGN serve as attachment receptors for SINV and

enhance viral infectivity when overexpressed in cells [37]. Interestingly, SINV derived from mosquito cells was better able to infect DC-SIGN- or L-SIGN-expressing cells than virus derived from mammalian cells, likely due to differential glycosylation of the viral envelope glycoproteins [37]. Similarly, RRV and VEEV produced from mosquito cells had greater infectivity for myeloid dendritic cells than mammalian cell-derived virus; however, whether this effect was due to interactions with a specific CLR has not been determined [38]. Unlike mammalian cell-derived RRV or VEEV, exposure of dendritic cells to mosquito cell-derived virus failed to induce a robust IFN response [38]. This was likely due to differences in host detection of viral glycans, as IFN induction was dependent upon the presence of the glycosylation sites in the RRV E2 glycoprotein [39]. These findings suggest that DC-SIGN, L-SIGN, and potentially other host CLRs respond differently to the viral glycosylation patterns generated in mosquito versus mammalian hosts.

CLRs also can regulate the inflammatory response to alphavirus infection. Mannose Binding Lectin (MBL), a soluble C-type lectin, promotes RRV disease as MBL A/C-deficient mice had reduced tissue damage following RRV infection relative to WT mice [40]. MBL enhanced expression of inflammatory mediators but had no impact on RRV replication in tissues or the recruitment of inflammatory cells to sites of infection [40]. In contrast, dendritic cell immune receptor (DCIR) serves as a negative regulator of inflammation, and *DCIR*^{-/-} mice infected with CHIKV had increased inflammatory disease and sustained viral replication [41]. Thus, the role of CLRs during alphavirus infection appears to be variable, ranging from protective to damaging responses.

Inhibitory effector proteins

The induction and secretion of type I IFN results in activation of a signaling cascade downstream of the type I IFN receptor that induces hundreds of interferon stimulated genes (ISGs). A subset of ISGs has been shown to impede alphavirus replication (Figure 2), and we discuss these in detail below. However, more work is needed to uncover additional ISGs that contribute to alphavirus control. To do this on a large scale, investigators have employed a variety of approaches. Several candidate inhibitory ISGs have been identified through large-scale ectopic expression studies, including new ISGs that may be important for alphavirus control (cGAS, PR2Y6, HPSE, NAMPT, MAP3K14, HES4, SAMD4A, TRIM14, IRF-1) [42,43]. In another overexpression approach, alphaviruses were engineered to express ISGs via a second subgenomic promoter to identify ISGs with robust inhibitory activity [44]. Building on this concept, a library of 10,000 SINVs was constructed with each virus engineered to express a unique artificial micro-RNA (amiRNA) that would target a murine open reading frame [45]. This approach allowed for *in vivo* screening, and passage through mice selected for viruses expressing amiRNAs targeting antiviral effectors. RNAi knockdown screens also have proven effective for identifying host factors that either obstruct or enhance alphavirus replication [46–48]. These approaches typically rely on cell culture studies, and candidates must be further validated to assess their role in the replication and pathogenesis of alphaviruses *in vivo* and to better characterize their mechanisms of action.

A few ISGs have been characterized during alphavirus infection. Protein Kinase R (PKR) inhibits translation in response to double-stranded RNA, and PKR is activated following

infection with SINV, SFV, and CHIKV [26,49–51]. PKR also enhances IFN mRNA stability during alphavirus infection, as IFN levels were decreased in PKR KO cells relative to WT cells following infection with SFV [51,52]. Despite this, SINV and SFV pathogenicity was unaltered in PKR-deficient mice, suggesting that PKR is not essential for control of these viruses *in vivo* [50,51]. SINV and SFV overcome PKR translational inhibition via a stem loop structure (downstream loop (DLP)) that directs initiation of translation of the subgenomic viral RNA [49]. This DLP is essential for replication of SINV in mammalian cell lines and mice, which express PKR, but is dispensable in mosquito cells, which lack PKR [53]. A homologous DLP has not been detected in a number of other alphavirus species, including CHIKV, WEEV, and VEEV [53]. Thus, it remains unclear if and how alphavirus species lacking a DLP overcome PKR.

PKR is not the only ISG that inhibits translation following alphavirus infection, as translational inhibition is observed during infection with SINV, SFV, or CHIKV in cells lacking PKR [26,54]. However, it remains unclear which ISG(s) contribute to this translational inhibition. One possibility is OAS3, as overexpression of OAS3 in HeLa cells inhibits CHIKV, SINV, and SFV replication [55]. OAS3 is a member of the 2′–5′-oligoadenylate synthetase (OAS) family of proteins, which produce 2′–5′-oligoadenylates in response to dsRNA. This activates RNase L, which cleaves viral and host ssRNAs to suppress translation [56]. One study suggested OAS3 activity during alphavirus infection was independent of RNase L, as a partial knockdown of RNase L in HeLa cells overexpressing OAS3 failed to relieve OAS3 inhibition [55]. However, a second study found that OAS3 was essential for activating RNase L during SINV infection in A549 cells [57]. Moreover, knockout of RNase L, OAS3, or the related OAS1 in A549 cells led to a moderate increase in SINV titers [57]. Despite this, SINV pathogenicity was not enhanced in mice lacking both PKR and RNase L [50].

IFIT1 distinguishes host mRNA from viral RNA by selectively inhibiting translation of RNAs lacking 2′-O-methylation at the 5′ end [58]. Unlike many cytoplasmic viruses that encode methyltransferases or snatch host caps, alphaviruses lack 2′-O-methylation and therefore should be sensitive to IFIT1. However, secondary structure elements in the 5′-UTR of VEEV and SINV inhibit IFIT1 binding [59]. Decreasing the stability of these structural elements restores IFIT1 interactions, allowing for inhibition of VEEV and SINV in the presence of IFIT1 [59]. Moreover, variability within the 5′ secondary structure of different strains and species of alphaviruses results in a range of susceptibilities to IFIT1 [60].

ZAP (PARP13) impedes translation of incoming alphavirus RNA and inhibits the replication of multiple alphaviruses when overexpressed in rat fibroblasts [61]. In addition, expression of ZAP from a second subgenomic promoter within the viral genome attenuated SINV in neonatal mice [44]. Mechanistically, ZAP synergizes with other ISGs for optimal antiviral activity [62,63]. For example, ZAP translational repression is lost in cells lacking TRIM25, an E3 ubiquitin and ISG15 ligase that interacts with ZAP [63]. Other members of the PARP family (PARP7, PARP10, and PARP12) also inhibit alphavirus replication *in vitro* by repressing translation [64,65]; however, their influence on alphavirus dissemination and pathogenicity *in vivo* has not been reported.

RSAD2, which encodes viperin, was highly induced in monocytes collected from CHIKV-infected patients. CHIKV replication was enhanced in *Rsad2*^{-/-} fibroblasts, and *Rsad2*^{-/-} mice infected with CHIKV had increased viremia and exhibited more severe swelling of the inoculated foot [66]. The inhibitory activity of viperin was dependent on ER localization, however the direct mechanism by which viperin inhibits CHIKV replication has not been fully elucidated [66].

MxA is an IFN-inducible GTP-ase. Stable expression of MxA in HEP-2 or U937 cells inhibited SFV replication, as evidenced by decreased viral titers and lower yields of viral protein and RNA [67]. The inhibitory activity of MxA was independent of the presence of SFV structural proteins, but beyond this the mechanism of action remains uncharacterized.

ISG15 plays a critical role in controlling alphaviruses, as expression of ISG15 from a second subgenomic promoter within SINV protected *Ifnar1*^{-/-} mice from lethality [68]. Consistent with this, *ISG15*^{-/-} mice were more susceptible to SINV and CHIKV [69,70]. Moreover, *ISG15*^{-/-} mice had markedly enhanced levels of proinflammatory cytokines and chemokines in response to CHIKV, with unaltered viral loads, suggesting ISG15 may function as an immunoregulator [70].

Membrane-associated ISGs also contribute to control of alphavirus infection. IFITM3 is a transmembrane protein that inhibits endosomal fusion of multiple alphaviruses. Replication of CHIKV, SFV, SINV, ONNV, and VEEV was enhanced in mouse fibroblasts lacking *Ifitm3* [71], while overexpression of IFITM3 in A549 cells restricted SFV and SINV replication [72]. Consistent with this, *Ifitm3*^{-/-} mice infected with CHIKV had increased tissue burdens at early times post infection and enhanced swelling in the inoculated foot. At the opposite end of the lifecycle, *Bst2*, which encodes tetherin, inhibits release of SFV and CHIKV [73]. The CHIKV protein nsP1 may antagonize tetherin activity by down-regulating *Bst2* expression [74]. However, tetherin still contributes to CHIKV control as *Bst2*^{-/-} mice had decreased expression of type I and II IFNs and increased replication at the site of inoculation, resulting in enhanced viremia [75].

Finally, an IFN-activated transcription factor, promyelocytic leukemia zinc finger (PLZF), inhibits alphaviruses. Pre-treatment of neonatal mice with IFN led to increased resistance to SFV in WT mice, while PLZF deficient mice (*Zbtb16*^{-/-}) remained sensitive and had elevated viral tissue burdens [76]. While serum IFN concentrations were not altered in *Zbtb16*^{-/-} mice, the expression of a subset of ISGs (including *Rsad2*, *OAS1*, and *IFIT2*) was impaired. Interestingly, both *Rasd2* and *OAS1* have been implicated in alphavirus control [57,66].

Concluding Remarks

While progress has been made, there are still many unanswered questions surrounding the innate immune response to alphavirus infection. TLR3, TLR7, and RLRs respond to alphavirus infection, yet it remains unclear which cell types they function within and whether these sensors are sufficient for a complete IFN response. Moreover, some anti-alphavirus ISGs have been identified, but more work is necessary to elucidate their

mechanisms of action and to uncover the full repertoire of ISGs that contribute to efficient control of alphaviruses. In addition, other components of the innate immune system contribute to alphavirus control, including CLRs and NLRs. However, our understanding of how these sensors, and likely others, impact alphavirus replication is limited. The significant burden of disease caused by alphaviruses and the lack of effective treatments adds urgency for an improved understanding of the mechanisms employed by the innate immune response to control alphavirus infection.

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References

1. Griffin, DE. Alphaviruses. In: Howley, PM.Griffin, DE.Lamb, RA.Knipe, DL.Martin, MA., Roizman, B., editors. *Fields' Virology*. Lippincott Williams & Wilkins; 2007.
2. Suhrbier A, Jaffar-Bandjee M-C, Gasque P. Arthritogenic alphaviruses—an overview. *Nature Reviews Rheumatology*. 2012; 8:420–429. [PubMed: 22565316]
3. Weaver SC, Forrester NL. Chikungunya: Evolutionary history and recent epidemic spread. *Antiviral Res*. 2015; 120:32–39. [PubMed: 25979669]
4. Cerny T, Schwarz M, Schwarz U, Lemant J, Gérardin P, Keller E. The Range of Neurological Complications in Chikungunya Fever. *Neurocritical Care*. 2017; doi: 10.1007/s12028-017-0413-8
5. Ronca SE, Dineley KT, Paessler S. Neurological Sequelae Resulting from Encephalitic Alphavirus Infection. *Front Microbiol*. 2016; 7:1216–10. [PubMed: 27540373]
6. Hwang SY, Hertzog PJ, Holland KA, Sumarsono SH, Tymms MJ, Hamilton JA, Whitty G, Bertoncello I, Kola I. A null mutation in the gene encoding a type I interferon receptor component eliminates antiproliferative and antiviral responses to interferons alpha and beta and alters macrophage responses. *PNAS*. 1995; 92:11284–11288. [PubMed: 7479980]
7. Müller U, Steinhoff U, Reis LF, Hemmi S, Pavlovic J, Zinkernagel RM, Aguet M. Functional role of type I and type II interferons in antiviral defense. *Science*. 1994; 264:1918–1921. [PubMed: 8009221]
8. Grieder FBEA. Role of Interferon and Interferon Regulatory Factors in Early Protection against Venezuelan Equine Encephalitis Virus Infection. 1999 [no volume].
9. Ryman KD, Klimstra WB, Nguyen KB, Biron CA, Johnston RE. Alpha/beta interferon protects adult mice from fatal Sindbis virus infection and is an important determinant of cell and tissue tropism. *Journal of Virology*. 2000; 74:3366–3378. [PubMed: 10708454]
10. Couderc T, Chrétien F, Schilte C, Disson O, Brigitte M, Guivel-Benhassine F, Touret Y, Barau G, Cayet N, Schuffenecker I, et al. A Mouse Model for Chikungunya: Young Age and Inefficient Type-I Interferon Signaling Are Risk Factors for Severe Disease. *PLoS Pathog*. 2008; 4:e29–12. [PubMed: 18282093]
11. Seymour RL, Rossi SL, Weaver SC, Bergren NA, Plante KS. The Role of Innate versus Adaptive Immune Responses in a Mouse Model of O'Nyong-Nyong Virus Infection. *Am J Trop Med Hyg*. 2013; 88:1170–1179. [PubMed: 23568285]
12. Stoermer Burrack KA, Hawman DW, Jupille HJ, Oko L, Minor M, Shives KD, Gunn BM, Long KM, Morrison TE. Attenuating Mutations in nsP1 Reveal Tissue-Specific Mechanisms for Control of Ross River Virus Infection. *Journal of Virology*. 2014; 88:3719–3732. [PubMed: 24429363]
13. Frangkoudis R, Breakwell L, McKimmie C, Boyd A, Barry G, Kohl A, Merits A, Fazakerley JK. The type I interferon system protects mice from Semliki Forest virus by preventing widespread virus dissemination in extraneural tissues, but does not mediate the restricted replication of avirulent virus in central nervous system neurons. *Journal of General Virology*. 2007; 88:3373–3384. [PubMed: 18024907]

14. White LJ, Wang JG, Davis NL, Johnston RE. Role of Alpha/Beta Interferon in Venezuelan Equine Encephalitis Virus Pathogenesis: Effect of an Attenuating Mutation in the 5' Untranslated Region. *Journal of Virology*. 2001; 75:3706–3718. [PubMed: 11264360]
15. Schilte C, Couderc T, Chrétien F, Sourisseau M, Gangneux N, Guivel-Benhassine F, Kraxner A, Tschopp J, Higgs S, Michault A, et al. Type I IFN controls chikungunya virus via its action on nonhematopoietic cells. *J Exp Med*. 2010; 207:429–442. [PubMed: 20123960]
16. Schilte C, Buckwalter MR, Laird ME, Diamond MS, Schwartz O, Albert ML. Cutting Edge: Independent Roles for IRF-3 and IRF-7 in Hematopoietic and Nonhematopoietic Cells during Host Response to Chikungunya Infection. *J I*. 2012; 188:2967–2971. Demonstrated that IRF3 and IRF7 are functionally redundant, and expression of IRF3/7 from either hematopoietic or nonhematopoietic cells is sufficient for CHIKV control.
17. Schlee M, Hartmann G. Discriminating self from non-self in nucleic acid sensing. *Nat Rev Immunol*. 2016; 16:566–580. [PubMed: 27455396]
18. Chen I-Y, Ichinohe T. Response of host inflammasomes to viral infection. *Trends in Microbiology*. 2015; 23:55–63. [PubMed: 25456015]
19. Monteiro J, Lepenies B. Myeloid C-Type Lectin Receptors in Viral Recognition and Antiviral Immunity. *Viruses*. 2017; 9:59–22.
20. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol*. 2004; 4:499–511. [PubMed: 15229469]
21. Rudd PA, Wilson J, Gardner J, Larcher T, Babarit C, Le TT, Anraku I, Kumagai Y, Loo YM, Gale M, et al. Interferon Response Factors 3 and 7 Protect against Chikungunya Virus Hemorrhagic Fever and Shock. *Journal of Virology*. 2012; 86:9888–9898. [PubMed: 22761364]
22. Neighbours LM, Long K, Whitmore AC, Heise MT. Myd88-Dependent Toll-Like Receptor 7 Signaling Mediates Protection from Severe Ross River Virus-Induced Disease in Mice. *Journal of Virology*. 2012; 86:10675–10685. [PubMed: 22837203]
23. Her Z, Teng TS, Tan JJ, Teo TH, Kam YW, Lum FM, Lee WW, Gabriel C, Melchioti R, Andiappan AK, et al. Loss of TLR3 aggravates CHIKV replication and pathology due to an altered virus-specific neutralizing antibody response. *EMBO Molecular Medicine*. 2015; 7:24–41. Demonstrated that TLR3 plays a protective role during CHIKV infection by limiting viral replication and enhancing the neutralizing antibody response. [PubMed: 25452586]
24. Esen N, Blakely PK, Rainey-Barger EK, Irani DN. Complexity of the Microglial Activation Pathways that Drive Innate Host Responses During Lethal Alphavirus Encephalitis in Mice. *ASN Neuro*. 2012; 4 AN20120016–15.
25. Wollish AC, Ferris MT, Blevins LK, Loo Y-M, Gale M Jr, Heise MT. An attenuating mutation in a neurovirulent Sindbis virus strain interacts with the IPS-1 signaling pathway in vivo. *Virology*. 2013; 435:269–280. [PubMed: 23084425]
26. White LK, Sali T, Alvarado D, Gatti E, Pierre P, Streblow D, DeFilippis VR. Chikungunya virus induces IPS-1-dependent innate immune activation and protein kinase R-independent translational shutoff. *Journal of Virology*. 2011; 85:606–620. [PubMed: 20962078]
27. Akhrymuk I, Frolov I, Frolova EI. Both RIG-I and MDA5 detect alphavirus replication in concentration-dependent mode. *Virology*. 2016; 487:230–241. [PubMed: 26550947]
28. Chen G, Shaw MH, Kim Y-G, Nuñez G. NOD-Like Receptors: Role in Innate Immunity and Inflammatory Disease. *Annu Rev Pathol Mech Dis*. 2009; 4:365–398.
29. Assunção o-Miranda I, Bozza MT, Da Poian AT. Pro-inflammatory response resulting from sindbis virus infection of human macrophages: Implications for the pathogenesis of viral arthritis. *J Med Virol*. 2010; 82:164–174. [PubMed: 19950249]
30. Chen W, Foo SS, Rulli NE, Taylor A, Sheng KC, Herrero LJ, Herring BL, Lidbury BA, Li RW, Walsh NC, et al. Arthritogenic alphaviral infection perturbs osteoblast function and triggers pathologic bone loss. *PNAS*. 2014; 111:6040–6045. [PubMed: 24733914]
31. Mokhtarian F, Wesselingh SL, Choi S, Maeda A, Griffin DE, Sobel RA, Grob D. Production and role of cytokines in the CNS of mice with acute viral encephalomyelitis. *J Neuroimmunol*. 1996; 66:11–22. [PubMed: 8964904]

32. Wesselingh SL, Levine B, Fox RJ, Choi S, Griffin DE. Intracerebral cytokine mRNA expression during fatal and nonfatal alphavirus encephalitis suggests a predominant type 2 T cell response. *J I.* 1994; 152:1289–1297.
33. Ng LFP, Chow A, Sun Y-J, Kwek DJC, Lim P-L, Dimatatac F, Ng L-C, Ooi E-E, Choo K-H, Her Z, et al. IL-1 β , IL-6, and RANTES as Biomarkers of Chikungunya Severity. *PLoS ONE.* 2009; 4:e4261–8. [PubMed: 19156204]
34. Ekchariyawat P, Hamel R, Bernard E, Wichit S, Surasombatpattana P, Talignani L, Thomas F, Choumet V, Yssel H, Desprès P, et al. Inflammasome signaling pathways exert antiviral effect against Chikungunya virus in human dermal fibroblasts. *INFECTION, GENETICS AND EVOLUTION.* 2015; 32:401–408.
35. Chen W, Foo S-S, Zaid A, Teng T-S, Herrero LJ, Wolf S, Tharmarajah K, Vu LD, van Vreden C, Taylor A, et al. Specific inhibition of NLRP3 in chikungunya disease reveals a role for inflammasomes in alphavirus-induced inflammation. *Nature Microbiology.* 2017; Demonstrated that NLR sensing and inflammasome activation contributes to alphavirus control *in vivo*. doi: 10.1038/s41564-017-0015-4
36. Geijtenbeek TBH, Gringhuis SI. Signalling through C-type lectin receptors: shaping immune responses. *Nat Rev Immunol.* 2009; 9:465–479. [PubMed: 19521399]
37. Klimstra WB, Nangle EM, Smith MS, Yurochko AD, Ryman KD. DC-SIGN and L-SIGN Can Act as Attachment Receptors for Alphaviruses and Distinguish between Mosquito Cell- and Mammalian Cell-Derived Viruses. *Journal of Virology.* 2003; 77:12022–12032. Demonstrated that CLRs can differentially interact with mosquito cell- versus mammalian cell-derived alphaviruses. [PubMed: 14581539]
38. Shabman RS, Morrison TE, Moore C, White L, Suthar MS, Hueston L, Rulli N, Lidbury B, Ting JPY, Mahalingam S, et al. Differential Induction of Type I Interferon Responses in Myeloid Dendritic Cells by Mosquito and Mammalian-Cell-Derived Alphaviruses. *Journal of Virology.* 2006; 81:237–247. [PubMed: 17079324]
39. Shabman RS, Rogers KM, Heise MT. Ross River Virus Envelope Glycans Contribute to Type I Interferon Production in Myeloid Dendritic Cells. *Journal of Virology.* 2008; 82:12374–12383. [PubMed: 18922878]
40. Gunn BM, Morrison TE, Whitmore AC, Blevins LK, Hueston L, Fraser RJ, Herrero LJ, Ramirez R, Smith PN, Mahalingam S, et al. Mannose Binding Lectin Is Required for Alphavirus-Induced Arthritis/Myositis. *PLoS Pathog.* 2012; 8:e1002586–14. [PubMed: 22457620]
41. Long KM, Whitmore AC, Ferris MT, Sempowski GD, McGee C, Trollinger B, Gunn B, Heise MT. Dendritic Cell Immunoreceptor Regulates Chikungunya Virus Pathogenesis in Mice. *Journal of Virology.* 2013; 87:5697–5706. [PubMed: 23487448]
42. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, Rice CM. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature.* 2011; 472:481–485. Demonstrated the utility of a large-scale over-expression screen and revealed multiple new ISGs that may inhibit alphaviruses. [PubMed: 21478870]
43. Schoggins JW, MacDuff DA, Imanaka N, Gainey MD, Shrestha B, Eitson JL, Mar KB, Richardson RB, Ratushny AV, Litvak V, et al. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. *Nature.* 2014; 505:691–695. [PubMed: 24284630]
44. Zhang Y, Burke CW, Ryman KD, Klimstra WB. Identification and Characterization of Interferon-Induced Proteins That Inhibit Alphavirus Replication. *Journal of Virology.* 2007; 81:11246–11255. Employed a unique tool to identify anti-alphavirus ISGs by engineering viruses to express ISGs from a second subgenomic promoter. [PubMed: 17686841]
45. Varble A, Benitez AA, Schmid S, Sachs D, Shim JV, Rodriguez-Barrueco R, Panis M, Crumiller M, Silva JM, Sachidanandam R, et al. An *in vivo* RNAi screening approach to identify host determinants of virus replication. *Cell Host & Microbe.* 2013; 14:346–356. Provides an *in vivo* screening approach to identify host genes that hinder alphavirus replication. [PubMed: 24034620]
46. Ooi YS, Stiles KM, Liu CY, Taylor GM, Kielian M. Genome-Wide RNAi Screen Identifies Novel Host Proteins Required for Alphavirus Entry. *PLoS Pathog.* 2013; 9:e1003835–10. [PubMed: 24367265]

47. Panda D, Rose PP, Hanna SL, Gold B, Hopkins KC, Lyde RB, Marks MS, Cherry S. Genome-wide RNAi Screen Identifies SEC61A and VCP as Conserved Regulators of Sindbis Virus Entry. *Cell Reports*. 2013; 5:1737–1748. [PubMed: 24332855]
48. Karlas A, Berre S, Couderc TERES, Varjak M, Braun P, Meyer M, Gangneux N, Karo-Astover L, Weege F, Raftery M, et al. A human genome-wide loss-of-function screen identifies effective chikungunya antiviral drugs. *Nature Communications*. 2016; 7:1–14.
49. Ventoso I, Sanz MA, Molina S, Berlanga JJ, Carrasco L, Esteban M. Translational resistance of late alphavirus mRNA to eIF2 α phosphorylation: a strategy to overcome the antiviral effect of protein kinase PKR. *Genes Dev*. 2006; 20:87–100. [PubMed: 16391235]
50. Ryman KD, White LJ, Johnston RE. Effects of PKR/RNase L-dependent and alternative antiviral pathways on alphavirus replication and pathogenesis. *Viral ...* 2002; 15:53–76.
51. Barry G, Breakwell L, Frangkoudis R, Attarzadeh-Yazdi G, Rodriguez-Andres J, Kohl A, Fazakerley JK. PKR acts early in infection to suppress Semliki Forest virus production and strongly enhances the type I interferon response. *Journal of General Virology*. 2009; 90:1382–1391. [PubMed: 19264662]
52. Schulz O, Pichlmair A, Rehwinkel J, Rogers NC, Scheuner D, Kato H, Takeuchi O, Akira S, Kaufman RJ, Reis e Sousa C. Protein Kinase R Contributes to Immunity against Specific Viruses by Regulating Interferon mRNA Integrity. *Cell Host & Microbe*. 2010; 7:354–361. [PubMed: 20478537]
53. Ventoso I. Adaptive changes in alphavirus mRNA translation allowed colonization of vertebrate hosts. *Journal of Virology*. 2012; 86:9484–9494. [PubMed: 22761388]
54. Ryman KD, Meier KC, Nangle EM, Ragsdale SL, Korneeva NL, Rhoads RE, MacDonald MR, Klimstra WB. Sindbis Virus Translation Is Inhibited by a PKR/RNase L-Independent Effector Induced by Alpha/Beta Interferon Priming of Dendritic Cells. *Journal of Virology*. 2005; 79:1487–1499. [PubMed: 15650175]
55. Bréhin A-C, Casadémont I, Frenkiel M-P, Julier C, Sakuntabhai A, Desprès P. The large form of human 2',5'-Oligoadenylate Synthetase (OAS3) exerts antiviral effect against Chikungunya virus. *Virology*. 2009; 384:216–222. [PubMed: 19056102]
56. Kristiansen H, Gad HH, Eskildsen-Larsen S, Desprès P, Hartmann R. The oligoadenylate synthetase family: an ancient protein family with multiple antiviral activities. *Journal of Interferon & Cytokine Research*. 2011; 31:41–47. [PubMed: 21142819]
57. Li Y, Banerjee S, Wang Y, Goldstein SA, Dong B, Gaughan C, Silverman RH, Weiss SR. Activation of RNase L is dependent on OAS3 expression during infection with diverse human viruses. *Proc Natl Acad Sci US A*. 2016; 113:2241–2246.
58. Diamond MS, Farzan M. The broad-spectrum antiviral functions of IFIT and IFITM proteins. *Nat Rev Immunol*. 2012; 13:46–57. [PubMed: 23237964]
59. Hyde JL, Gardner CL, Kimura T, White JP, Liu G, Trobaugh DW, Huang C, Tonelli M, Paessler S, Takeda K, et al. A Viral RNA Structural Element Alters Host Recognition of Nonself RNA. *Science*. 2014; 343:783–787. Demonstrated that a structural element in the 5'UTR of alphaviruses antagonizes IFIT1 restriction. [PubMed: 24482115]
60. Reynaud JM, Kim DY, Atasheva S, Rasaloukaya A, White JP, Diamond MS, Weaver SC, Frolova EI, Frolov I. IFIT1 Differentially Interferes with Translation and Replication of Alphavirus Genomes and Promotes Induction of Type I Interferon. *PLoS Pathog*. 2015; 11:e1004863–31. [PubMed: 25927359]
61. Bick MJ, Carroll JWN, Gao G, Goff SP, Rice CM, MacDonald MR. Expression of the Zinc-Finger Antiviral Protein Inhibits Alphavirus Replication. *Journal of Virology*. 2003; 77:11555–11562. [PubMed: 14557641]
62. Karki S, Li MMH, Schoggins JW, Tian S, Rice CM, MacDonald MR. Multiple Interferon Stimulated Genes Synergize with the Zinc Finger Antiviral Protein to Mediate Anti-Alphavirus Activity. *PLoS ONE*. 2012; 7:e37398–13. [PubMed: 22615998]
63. Li MMH, Lau Z, Cheung P, Aguilar EG, Schneider WM, Bozzacco L, Molina H, Buehler E, Takaoka A, Rice CM, et al. TRIM25 Enhances the Antiviral Action of Zinc-Finger Antiviral Protein (ZAP). *PLoS Pathog*. 2017; 13:e1006145–25. [PubMed: 28060952]

64. Atasheva S, Akhrymuk M, Frolova EI, Frolov I. New PARP gene with an anti-alphavirus function. *Journal of Virology*. 2012; 86:8147–8160. [PubMed: 22623789]
65. Atasheva S, Frolova EI, Frolov I. Interferon-Stimulated Poly(ADP-Ribose) Polymerases Are Potent Inhibitors of Cellular Translation and Virus Replication. *Journal of Virology*. 2014; 88:2116–2130. [PubMed: 24335297]
66. Teng T-S, Foo S-S, Simamarta D, Lum F-M, Teo T-H, Lulla A, Yeo NKW, Koh EGL, Chow A, Leo Y-S, et al. Viperin restricts chikungunya virus replication and pathology. *J Clin Invest*. 2012; 122:4447–4460. [PubMed: 23160199]
67. Landis H, Simon-Jödicke A, Klöti A, Di Paolo C, Schnorr JJ, Schneider-Schaulies S, Hefti HP, Pavlovic J. Human MxA protein confers resistance to Semliki Forest virus and inhibits the amplification of a Semliki Forest virus-based replicon in the absence of viral structural proteins. *Journal of Virology*. 1998; 72:1516–1522. [PubMed: 9445055]
68. Lenschow DJ, Giannakopoulos NV, Gunn LJ, Johnston C, O'Guin AK, Schmidt RE, Levine B, Virgin HW. Identification of Interferon-Stimulated Gene 15 as an Antiviral Molecule during Sindbis Virus Infection In Vivo. *Journal of Virology*. 2005; 79:13974–13983. [PubMed: 16254333]
69. Lenschow DJ, Lai C, Frias-Staheli N, Giannakopoulos NV, Lutz A, Wolff T, Osiak A, Levine B, Schmidt RE, García-Sastre A, et al. IFN-stimulated gene 15 functions as a critical antiviral molecule against influenza, herpes, and Sindbis viruses. *PNAS*. 2007; 104:1371–1376. [PubMed: 17227866]
70. Werneke SW, Schilte C, Rohatgi A, Monte KJ, Michault A, Arenzana-Seisdedos F, Vanlandingham DL, Higgs S, Fontanet A, Albert ML, et al. ISG15 Is Critical in the Control of Chikungunya Virus Infection Independent of UbE1L Mediated Conjugation. *PLoS Pathog*. 2011; 7:e1002322–14. [PubMed: 22028657]
71. Poddar S, Hyde JL, Gorman MJ, Farzan M, Diamond MS. The Interferon-Stimulated Gene IFITM3 Restricts Infection and Pathogenesis of Arthritogenic and Encephalitic Alphaviruses. *Journal of Virology*. 2016; 90:8780–8794. [PubMed: 27440901]
72. Weston S, Czieso S, White IJ, Smith SE, Wash RS, Diaz-Soria C, Kellam P, Marsh M. Alphavirus Restriction by IFITM Proteins. *Traffic*. 2016; 17:997–1013. [PubMed: 27219333]
73. Ooi Y, Dubé M, Kielian M. BST2/Tetherin Inhibition of Alphavirus Exit. *Viruses*. 2015; 7:2147–2167. [PubMed: 25912717]
74. Jones PH, Maric M, Madison MN, Maury W, Roller RJ, Okeoma CM. BST-2/tetherin-mediated restriction of chikungunya (CHIKV) VLP budding is counteracted by CHIKV non-structural protein 1 (nsP1). *Virology*. 2013; 438:37–49. [PubMed: 23411007]
75. Mahauad-Fernandez WD, Jones PH, Okeoma CM. Critical role for bone marrow stromal antigen 2 in acute Chikungunya virus infection. *Journal of General Virology*. 2014; 95:2450–2461. [PubMed: 25053563]
76. Xu D, Holko M, Sadler AJ, Scott B, Higashiyama S, Berkofsky-Fessler W, McConnell MJ, Pandolfi PP, Licht JD, Williams BRG. Promyelocytic Leukemia Zinc Finger Protein Regulates Interferon-Mediated Innate Immunity. *Immunity*. 2009; 30:802–816. [PubMed: 19523849]

HIGHLIGHTS

- The Type I IFN response is essential for efficient control of alphaviruses.
- A number of pathogen recognition receptors (PRRs) and IFN-stimulated effector proteins have been shown to contribute to alphavirus control.
- The relative contributions of host PRRs during alphavirus infections and the cell types they function within *in vivo* are poorly understood.
- An understanding of the full repertoire of anti-alphavirus effector proteins and their mechanisms of action requires further research.

Figure 1. Host sensing of alphavirus infection

Following infection, multiple host sensors have been shown to respond to alphavirus infection. The alphavirus genome and replicative intermediates can engage Toll-like-receptor 3 (TLR3), TLR7, and the RIG-I-like receptors RIG-I and MDA5. These sensors signal through the downstream adaptor proteins MyD88, TRIF, or MAVS to activate a signaling cascade that results in activation of transcription factors IRF3 and IRF7, which translocate to the nucleus and induce type I IFN. Several C-type lectin receptors (CLRs) have been reported to respond to alphaviruses. DC-SIGN and L-SIGN can enhance alphavirus entry. DCIR responds to alphaviruses by decreasing inflammation, although the signaling pathway by which this occurs is not understood. MBL, a soluble c-type lectin, increases inflammatory mediators in response to alphaviruses. Nod-like receptors are also engaged by alphaviruses, although the activating signal is not known. NLRP3, and likely other NLRs, can be activated during alphavirus infection, driving the formation of an inflammasome composed of NLRP3, ASC, and caspase-1. Activation of caspase-1 by the inflammasome allows for proteolytic cleavage of pro-IL-1 β , resulting in secretion of IL-1 β .

Figure 2. Interferon-stimulated effector proteins with anti-alphavirus activity

In order to productively infect a cell, alphaviruses must enter through receptor-mediated endocytosis and fuse with the endosomal membrane to release the capsid and genomic RNA. The genome is then translated, allowing for synthesis of viral RNAs, including the genomic and the subgenomic mRNA, which is translated into structural proteins that assemble with the genomic RNA and bud from the cell membrane to release infectious progeny. Multiple steps of this lifecycle can be inhibited by interferon-stimulated genes (ISGs). Membrane bound proteins inhibit endosomal fusion (IFITM3) and virus release (tetherin). Alphavirus translation is inhibited by several ISGs. IFIT1 interacts with the 5' cap to block translation initiation, while PKR and OAS3/RNaseL are activated by dsRNA to globally inhibit translation. ZAP and other PARP family members inhibit translation in response to alphaviruses, but the mechanisms by which this occurs are not understood. The ISGs MxA, Viperin, and ISG15 also inhibit alphaviruses through unknown mechanisms. Lastly, PLZF, an IFN stimulated transcription factor, drives the expression of a subset of ISGs to inhibit alphavirus replication.