Genetic control of alphavirus pathogenesis

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Abstract
Alphaviruses, members of the positive-sense, single-stranded RNA virus family *Togaviridae*, represent a re-emerging public health concern worldwide as mosquito vectors expand into new geographic ranges. Members of the alphavirus genus tend to induce clinical disease characterized by rash, arthralgia, and arthritis (chikungunya virus, Ross River virus, and Semliki Forest virus) or encephalomyelitis (eastern equine encephalitis virus, western equine encephalitis virus, and Venezuelan equine encephalitis virus), though some patients who recover from the initial acute illness may develop long-term sequelae, regardless of the specific infecting virus. Studies examining the natural disease course in humans and experimental infection in cell culture and animal models reveal that host genetics play a major role in influencing susceptibility to infection and severity of clinical disease. Genome-wide genetic screens, including loss of function screens, microarrays, RNA-sequencing, and candidate gene studies, have further elucidated the role host genetics play in the response to virus infection, with the immune response being found in particular to majorly influence the outcome. This review describes the current knowledge of the mechanisms by which host genetic factors influence alphavirus pathogenesis and discusses emerging technologies that are poised to increase our understanding of the complex interplay between viral and host genetics on disease susceptibility and clinical outcome.

Introduction

Alphaviruses are a genus of enveloped, positive-sense, single-stranded RNA viruses belonging to the *Togaviridae* family (Kuhn 2007; Strauss and Strauss 1994). Alphavirus virions are approximately 70 nm in size and consist of capsid proteins surrounding a single RNA genome and two transmembrane glycoproteins, E1 and E2, which facilitate entry into cells by clathrin-mediated endocytosis (DeTulleo and Kirchhausen 1998). The genome ranges from 11 to 12 kb in length and contains a 5′methylguanylate cap and 3′polyadenylated tail and encodes both structural and nonstructural proteins. The four nonstructural proteins (nsp1, nsp2, nsp3, and nsp4) are encoded at the 5′ end; the five structural proteins, which include the capsid and E1 and E2 glycoproteins, are encoded at the 3′ end and translated from a subgenomic RNA. As a positive-sense RNA virus, the alphaviral genome is infectious, meaning when introduced into a permissive cell, the RNA can automatically replicate and produce infectious virus particles.

Most alphavirus members are transmitted by arthropods, specifically mosquitoes, which make the alphaviruses a re-emerging public health threat as arthropod vectors expand into new territories. The natural sylvatic cycle occurs between mosquitos and bird, rodent, or nonhuman primate (NHP) reservoirs, but occasionally promiscuous mosquitos feed on larger mammals such as humans, inducing clinical disease (Griffin 2013). Other alphaviruses are horizontally transmitted without an insect vector, such as salmon alphavirus (SAV), which causes pancreas disease in salmon and trout; SAV is horizontally transmitted primarily through water and responsible for massive economic losses during outbreaks in commercial fish populations in Northern Europe (Aunsmo et al. 2010; McLoughlin and Graham 2007).

Arthropod-borne alphaviruses are found worldwide and are generally divided into two major groups based on the

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disease syndrome that typically manifests in humans during natural infection (Ryman and Klimstra 2008). The Old World alphaviruses, which include Sindbis virus (SINV), chikungunya virus (CHIKV), Semliki Forest virus (SFV), and Ross River virus (RRV), are generally found in Europe, Africa, Asia, and Oceania and typically produce a clinical syndrome characterized by fever, rash, and arthritis (Hollidge et al. 2010). In contrast, the New World alphaviruses, which include eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV), and Venezuelan equine encephalitis virus (VEEV), are naturally found in North and South America, and induce encephalomyelitis in humans. However, it is important to note that atypical disease manifestations are sometimes reported, such as encephalitis in individuals infected with CHIKV or SFV (Ganesan et al. 2008; Willems et al. 1979).

Mortality rates following infection with arthropod-borne alphaviruses vary depending on the virus species; Old World alphaviruses such as CHIKV rarely cause death, while some New World alphaviruses, such as EEEV, have mortality rates reported to be as high as 70% in symptomatic individuals (Steele et al. 2007). Alphaviruses are typically considered to cause an acute disease process in humans, but reports of chronic debilitating sequelae are not uncommon. This includes myalgia and arthralgia lasting months to years following infection with Old World alphaviruses (Alla and Combe 2011; Hawman et al. 2013; Sane et al. 2012) and lifelong neurological deficits in individuals infected with New World alphaviruses (Bruyn and Lennette 1953; Palmer and Finley 1956; Villari et al. 1995). No vaccines against alphaviruses are currently available for civilian use in humans, and treatment is limited to supportive care (Go et al. 2014; Griffin 2010). This propensity to cause lasting physical debilitations that can induce significant physical, emotional, and financial disability makes it increasingly important to understand the pathogenesis of alphavirus-induced disease so that better preventatives and treatments may be developed.

Understanding the pathogenesis of alphavirus infection has been greatly advanced thanks to animal models. As obligate intracellular parasites, alphaviruses require finding a permissive cell in order to replicate within and then exit in order to move to a new location. In order to cause disease, a virus must enter the host (usually through a mosquito bite for alphaviruses), replicate locally, and then disseminate to target organs. For the Old World alphaviruses, those target organs are mostly skin, joints, and muscle during acute infection. For the New World alphaviruses, those target organs are the brain and spinal cord. While natural strains of the Old World alphavirus SINV tend to induce fever and arthritis in humans (Adouchief et al. 2016), strains used for experimental infection are more neurotropic, making SINV the prototypic alphavirus for examining infection of the CNS (Dubuisson et al. 1997). The well-characterized mouse model of alphavirus encephalomyelitis using SINV has helped elucidate the pathogenesis of CNS alphaviral infection and the role the immune response plays in both inducing CNS damage and facilitating virus clearance (Baxter et al. 2017; Griffin et al. 1992).

Virus entry into the CNS requires bypassing the blood brain barrier. Most neurotropic viruses enter the CNS via one of three methods: (1) hematogenous entry via infection of endothelial cells, (2) hematogenous entry through breakdown of the BBB; (3) access to the CNS by routes other than through the BBB, such as through peripheral nerves or the blood-cerebrospinal fluid (CSF) barrier, and (4) through virus-infected leukocytes that naturally cross the BBB ("Trojan horse" model) (Swanson and McGavern 2013). WEEV, VEEV, and the SINV model of New World alphavirus infection enter the CNS through hematogenous spread to circumventricular organs that lack a normal BBB or via axonal transport via olfactory sensory or peripheral neurons (Charles et al. 1995; Phillips et al. 2016; Passoni et al. 2017; Thach et al. 2001). In contrast, EEEV and neuroinvasive CHIKV have been shown to enter the brain primarily through a vascular route (Honnold et al. 2015; Passoni et al. 2017; Vogel et al. 2005). Following intranasal VEEV infection, pro-inflammatory cytokines IFN-β, TNF-α, and IL-6 mediate the breakdown of the BBB in conjunction with monocyte infiltration into the brain, allowing for a second wave of neuroinvasion by the virus, and inhibition of BBB opening by using a MMP-9 inhibitor results in delayed disease onset (Cain et al. 2017; Schäfer et al. 2011). Mice deficient in ICAM-1, an adhesion molecule important in regulating leukocyte transmigration across blood vessels, show reduced severity of clinical disease and improved survival with reduced perivascular cuffing and downregulation of pro-inflammatory cytokine expression following VEEV infection, suggesting transmigration of leukocytes across the blood brain barrier is important in clinical disease development (Sharma et al. 2011).

Both the innate and adaptive immune response have been shown to play a major role in the pathogenesis of both the Old World and New World alphaviruses via both protective and pathologic mechanisms. Synovial effusions of humans infected with RRV and CHIKV are characterized by monocytes/macrophages and CD4 + T cells, (Fraser et al. 1981; Hoarau et al. 2010; Rulli et al. 2007). Muscle biopsies from patients naturally infected with CHIKV have shown variable infiltration of macrophages and T cells, and histological changes to the muscle tissue include atrophy, vacuolization, and necrosis in muscle fibers (Muelas et al. 2017; Ozden et al. 2007). Nonhuman primate and mouse models of CHIKV and RRV infection show extensive infiltration of mononuclear cells into lymphoid tissues, muscle, and synovial tissues (Couderc et al. 2008; Labadie et al.

2010; Morrison et al. 2006, 2011). Histological changes of CNS tissue of humans and horses infected with New World alphaviruses are characterized by massive infiltration of neutrophils early in the disease process, but as infection progresses, lymphocytes soon replace them as the predominant immune cell population (Greenlee 2014; Hatanpaa and Kim 2014). In the mouse model of fatal alphavirus encephalomyelitis using the NSV strain of SINV, pathological changes include marked loss of neurons, particularly hippocampal neurons of the brain and motor neurons of the brainstem and spinal cord, accompanied by perivascular cuffing and parenchymal infiltration of mononuclear cells (Kimura and Griffin 2003). The character, magnitude, and timing of the immune response are highly influenced by host genetics, and genetic variability within and between populations results in differences in this immune response to virus infection, affecting infection susceptibility and disease outcome.

Natural genetic variation can affect disease outcome and susceptibility through a variety of mechanisms. Changes in noncoding promoter or repressor regions, 3′ and 5′ UTRs, coding sequences, and gene splice sites can result in a variety of differences in gene and protein expression and functionality. Multiple changes to the same gene can be found within an individual or population, and these genetic changes both individually and collectively can influence the response to a virus infection. While some genetic variants may result in an extreme phenotype, such as individuals homozygous for a defective CCR5 chemokine receptor being highly resistant to HIV infection (Huang et al. 1996), most result in much more moderate or subtle differences. The combined interaction of environmental, demographic, and genetic influences plays a major role in an individual or population's susceptibility to developing disease.

Host genetics can impact different aspects of virus infec-tion and resulting disease. Genetic mutations to receptor proteins can affect a virus's ability to infect its target cells, such as "non-secretor" individuals with a homozygous non-sense mutation in the *FUT2* gene being resistant to norovi-rus infection of the gut epithelium (Le Pendu et al. 2006). Genetic variability can also alter the disease manifestation, severity, or outcome once a successful infection is obtained, such as children with certain SNPs in innate immune genes *VDR, IFNA5*, and *NOS2* being more likely to develop bron-chiolitis requiring hospitalization following respiratory syncytial virus infection (Janssen et al. 2007). Our current understanding of the role host genetics plays in alphavirus pathogenesis is fairly limited despite evidence indicating host genetic variability impacts development and severity of clinical disease. Subclinical infections are extremely com-mon for many alphaviruses, and incidence of neurological disease development in adults following successful infec-tion with EEEV and WEEV has been found to be less than 5% and 0.1%, respectively (Calisher 1994; Goldfield et al. 1968). During two different CHIKV outbreaks associated with a high incidence of CNS infection, specific neurological disease manifestations largely differed despite the causative viruses belonging to the same phylogenetic group; in La Reunion, encephalitis was most frequently reported, while in India, peripheral neuropathy with a presumed underlying autoimmune mechanism was most commonly reported (Cerny et al. 2017). This review summarizes our current understanding of the role that host genetic factors play in alphavirus pathogenesis through both genome-wide genetic approaches and candidate gene studies. We also discuss emerging technologies that are poised to rapidly expand our understanding of how genetic variation impacts disease susceptibility and outcome to alphavirus infection.

Alphavirus pathogenesis in humans

Most of our understanding of the genetic control of alphavirus pathogenesis in humans comes from case reports and studies of Old World alphaviruses, particularly CHIKV. The incubation period for CHIKV is generally thought to last from 3 to 7 days, at which point symptoms typically start with a biphasic fever that can last up to 2 weeks (Staples et al. 2009). Debilitating symmetrical polyarthralgia most commonly affecting the wrists, elbows, fingers, knees, and ankles soon follows. Maculopapular rash on the trunk and extremities appearing with the onset of fever is a more variable finding; other common symptoms during the acute phase include headache, fatigue, nausea, conjunctivitis, and myalgia.

Several case reports and studies have examined the presence of biomarkers in serum or plasma during acute and convalescent CHIKV infection. Early in the clinical phase of acute infection, pro-inflammatory cytokines and chemokines are commonly elevated, including interleukins (IL) 1, 6, 7, 12, 13, 15, 17, and 18, tumor necrosis factor alpha (TNF-α), interferons alpha (IFN-α) and gamma (IFN-γ), granulocyte–macrophage colony-stimulating factor (GM-CSF), granulocyte-colony-stimulating factor (G-SCF), monocyte chemoattractant protein 1 (MCP-1/CCL2), CXCL9, and CXCL10 (Chaaitanya et al. 2011; Chirathaworn et al. 2013; Chopra et al. 2014; Chow et al. 2011; Kelvin et al. 2011; Lohachanakul et al. 2012; Reddy et al. 2014; Teng et al. 2015; Venugopalan et al. 2014; Wauquier et al. 2011); elevated IL-10 is a more variably reported finding (Chaaitanya et al. 2011). Levels of these pro-inflammatory cytokines are associated with a higher CHIKV load in the serum, as is lymphopenia, decreased monocyte numbers, and neutrophilia (Chow et al. 2011; Reddy et al. 2014; Teng et al. 2015). Higher IL-1β, IL-6, and IL-8 levels but lower RANTES levels in the serum are associated with more severe disease during the acute phase (Lohachanakul et al.

2012; Ng et al. 2009). Elevated serum mannose-binding lectin (MBL), a pattern recognition receptor involved in activation of the complement system, correlates with disease severity in individuals infected with RRV, another Old World alphavirus (Gunn et al. 2012). During the recovery phase of CHIKV infection, many pro-inflammatory cytokines continue to be elevated, though at lower levels than during acute infection; this includes IL-6, IL-13, GM-CSF, CCL2, CCL4, CXCL10 (Chirathaworn et al. 2013; Chopra et al. 2014). Other cytokines, such as IL-1β, IL-5, IL-12, IL-10, IL-18, TNF-α, and IFN-γ, have been reported to be at higher levels in convalescent serum compared to acute phase serum (Chirathaworn et al. 2010; Kelvin et al. 2011).

Chronic arthralgia is a common sequelae of CHIKV infection and has been reported in up to 68% of patients over a year following recovery from acute febrile illness (Borgherini et al. 2008; Chang et al. 2017; Gauri et al. 2016; Gérardin et al. 2011; Sissoko et al. 2009). Development of chronic arthralgia is more commonly seen in older patients, females, and individuals with co-morbidities, especially diabetes mellitus (Badawi et al. 2018; Elsinga et al. 2017; Heath et al. 2018). The chronic phase of infection has been characterized by continued elevation of pro-inflammatory levels of IL-6, IL-8, GM-CSF, CCL2, CCL3, CCL4, CXCL9, and CXCL10 (Chaaitanya et al. 2011; Chow et al. 2011; Kelvin et al. 2011; Reddy et al. 2014). Compared to patients who completely recover from CHIKV infection, individuals with persistent arthralgia and arthritis have an anti-viral immune response characterized by peripheral blood mononuclear cells (pBMCs) possessing high levels of IFN-α mRNA, high numbers of TNF-α and IFN-γ-secreting NKT cells, and high circulating levels of IL-6 and IL-12 (Hoarau et al. 2010; Sepúlveda-Delgado et al. 2017; Thanapati et al. 2017). A study performed during the 2008 CHIKV outbreak in Singapore found that IgG3 dominated the CHIKV-specific antibody response, and while patients who developed high IgG3 levels during the acute phase initially developed more severe febrile disease, they had faster virus clearance and were less likely to develop chronic arthralgia; in contrast, low viremia and a delayed IgG3 response were associated with persistent arthralgia (Kam et al. 2012). Hyperferritinemia during the acute phase of infection was positively associated with chronic arthralgia in patients examined during the 2014–2015 CHIKV outbreak in Curacao (Anfasa et al. 2017). Atypical disease manifestations, particularly neurological symptoms, have been reported in CHIKV patients. Neurological CHIKV infection has been associated with increases of TNF-α, IFN-α, IL-6, IL-8, CCL2, CCL5/RANTES, CCL17, and CXCL9 in the cerebrospinal fluid (CSF), with elevation of IL-6 and IL-8 in the CSF compared to the serum correlating to neurological involvement (Kashyap et al. 2014). Identifying biomarkers that can predict the development of chronic disease provide an opportunity for early treatment intervention that can hopefully mitigate chronic symptom development.

A few studies have examined genetic susceptibility behind alphavirus-induced clinical disease, particularly polymorphisms involving specific components of the immune response. Human leukocyte antigens (HLA) play a major role in adaptive immune response initiation and have been reported to be associated with disease outcome (reviewed in Dendrou et al. 2018). Polymorphisms in genes encoding HLA class II molecules, which present peptides to CD4 + T cells, were examined to determine the effect on susceptibility or protection against CHIKV-induced disease (Chaaithanya et al. 2013); HLA-DQ molecules were found to bind more CHIKV peptides than HLA-DRB1 molecules, and HLA-DQB1*03:03 and other HLA-DQB1 genotypes containing glutamic acid at position 86 of peptide-binding pocket 1 were found at a lower frequency in CHIKV patients compared to the control population. Toll-like receptors (TLRs) that recognize viral RNA genomes, including TLR-3, TLR-7, and TLR-8, are key activators of the innate immune response, including pro-inflammatory cytokine induction. Examination of single-nucleotide polymorphisms (SNPs) of TLR-7 and TLR-8 revealed that the rs179010-CC, rs3853839-GC, and rs3853839-CC genotypes for TLR7 and the GC genotype for the rs3764879 polymorphism of TLR8 were associated with enhanced CHIKV susceptibility to disease (Dutta and Tripathi 2017). In a study examining CHIKV prevalence in blood donations in Guadeloupe and Martinique, increased seroprevalence was positively associated with A, Rhesus positive, Kell negative blood group in Martinique (Gallian et al. 2017); while the mechanisms behind this association are not known, the study authors speculated that individuals with these blood groups may have differential innate immune responses involved in virus elimination or that biological, epidemiological, or sociological effects influencing susceptibility to mosquito bites might be driving this outcome. Genetic variation related to the immune response can also affect disease outcome. During infection with RRV, patients carrying the G allele of the IL-6-174 G/C SNP, which is associated with high cytokine production, demonstrated poorer neurocognitive performance during acute sickness response (Cvejic et al. 2014).

Effect of host genetics on experimental alphavirus infection

Genome-wide genetic screens

Several genome-wide genetic screens have been used to study the pathogenesis of alphaviruses. Genome-wide screens using RNA-mediated interference (RNAi) have in particular been used to identify genes involved in SINV

entry and replication using a *Drosophila* system (Fig. 1a). NRAMP, a metal ion transporter found on host cell surfaces, was found to be required for SINV binding and entry into *Drosophila* cells by siRNA screen, with dNRAMP mutant flies and mammalian homolog NRAMP2-deficient murine cells resistant to SINV infection (Rose et al. 2011). SEC61A and valosin-containing protein (VCP) regulate trafficking of NRAMP2 to the cell surface, and depletion of these proteins via dsRNA treatment in *Drosophila* cells significantly impairs SINV infection (Panda et al. 2013). Another siRNA screen found that Nup98, involved in antiviral gene induction, cooperates with the transcription factor FoxK to regulate gene expression and thus restrict SINV replication in both *Drosophila* cells and adult flies, and siRNA depletion of the mammalian homolog FOXK1 in human HEK-293T cells increases SINV infection (Panda et al. 2015).

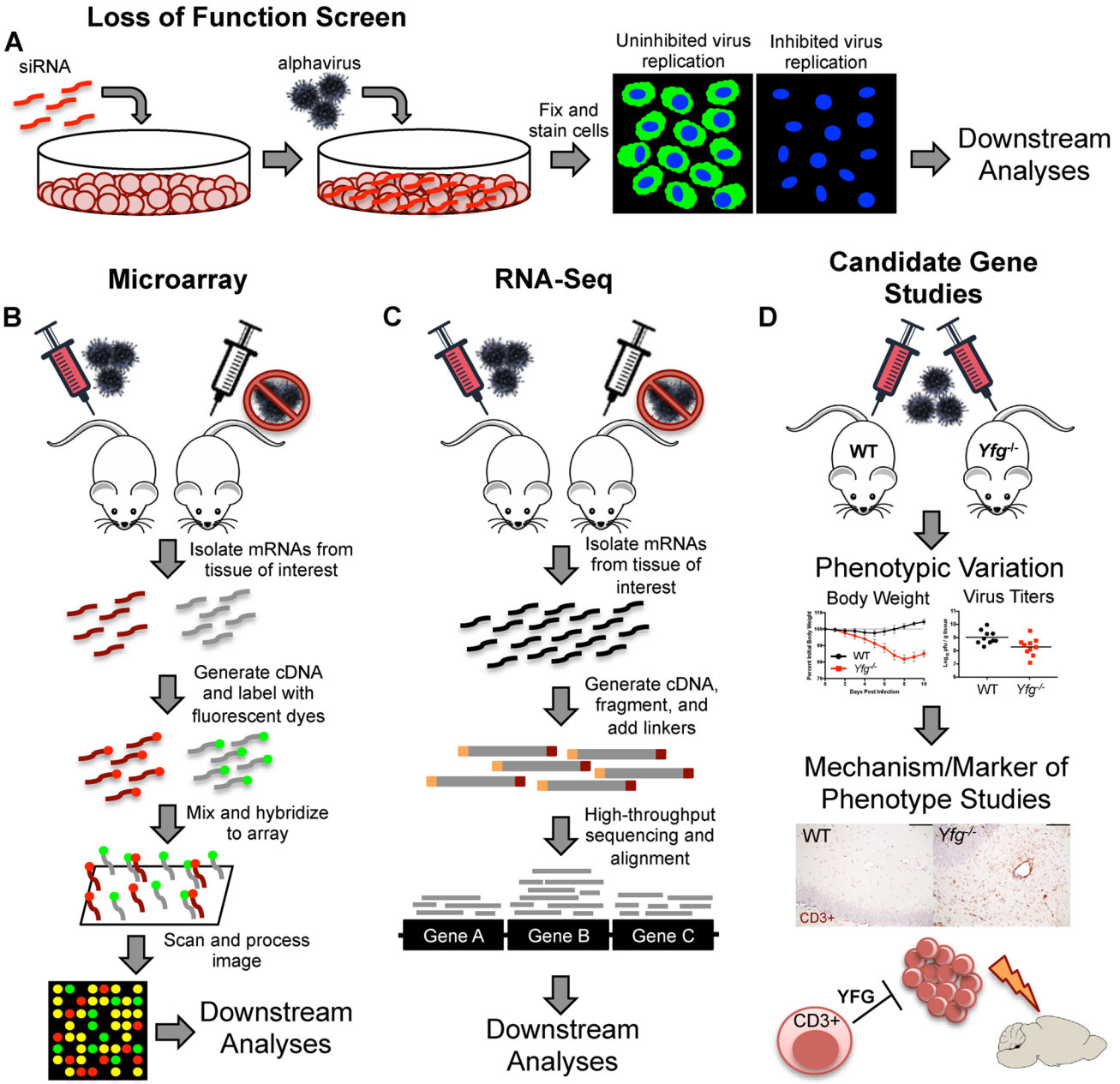

Fig. 1 Approaches for studying the role of host genetics on alphavirus pathogenesis. **a** Loss of function screen using siRNA to knockdown host gene expression. **b** Examination of differential gene expression in alphavirus-infected versus mock-infected mouse tissues using microarray. **c** Quantification of relative amounts of transcripts in alphavirus-infected versus mock-infected mouse tissues using RNA-sequencing (RNA-Seq). **d** Examination of the effect of individual genes on alphavirus pathogenesis through candidate gene studies. *YFG* your favorite gene

A genome-wide RNAi screen of West Nile virus infection using the *Drosophila* system found 50 genes conserved across invertebrates and vertebrates that restricted virus replication, with dXPO1 and dRUVBL1, part of the chromatin-remodeling Tip60 complex that regulates transcription, contributing to antiviral defense following infection with a wide variety of viruses, including SINV (Yasunaga et al. 2014).

siRNA screens have also been used to identify host factors that promote or inhibit infection exclusively in mammalian cells. A genome-wide siRNA screen examining susceptibility to SINV, SFV, and CHIKV infection in human cells identified FUZ, involved in cell polarity and mammalian embryonic development, and TSPAN9, involved in membrane organization and cellular adhesion, as novel host factors that promote alphavirus entry into cells (Ooi et al. 2013). Genes involved in selective autophagy during SINV infection have also been identified through RNAi screens, with murine embryonic fibroblasts (MEFs) deficient in *Smurf1* showing increased SINV capsid levels following infection (Orvedahl et al. 2011). A high-content imaging-based siRNA screen of VEEV-infected HeLa cells identified an actin-remodeling pathway involving RAC1, PIP5K1-α, and Arp3 important in alphavirus infection, with Rac1 and Arp3 essential for E2 trafficking from the trans-Golgi network to the cell surface via actin remodeling (Radoshitzky et al. 2016). A genome-wide loss of function screen performed with siRNAs in CHIKV-infected HEK-293T cells identified 156 pro-viral and 41 antiviral factors, and generation of cells deficient for 16 of these pro-viral factors using CRISPR/Cas9 identified post-translation modification, cellular function and maintenance, and RNA post-transcriptional modification as major pathways for future therapeutic targeting (Karlas et al. 2016). Another study used siRNA delivery through replication competent SINV constructs to perform RNAi screens in C57BL/6 mice and identified effectors that directly inhibited virus infection and indirectly inhibited virus infection through modulation of the antiviral response, such as Mga, which plays an important role in stimulating IFN-β production and thus inducing type I IFN ISG expression (Varble et al. 2013).

Whole-genome microarrays have also been performed to evaluate genes, particular immune response genes, and microRNAs (miRNA) involved in alphavirus pathogenesis (Fig. 1b). Transcriptome analysis of whole blood samples from human patients immunized with the TC-83 strain of VEEV revealed that early in infection, upregulated pathways are dominated by the innate sensing and immune response, including classic interferon response and associated factors, activation of PRRs, and engagement of the inflammasome (Erwin-Cohen et al. 2017). Later in the infection process at 14 days, major upregulated pathways include oxidative phosphorylation, protein ubiquitination, and immune processes such as NK cell signaling and B cell development (Erwin-Cohen et al. 2017). A microarray performed on mice infected with either a highly neurovirulent V3000 strain of VEEV or the partially neurovirulent V3034 strain of VEEV revealed V3000 induced a stronger inflammatory and apoptotic response, with expression of *Ccl2, Ccl5, Ccl6*, and *Ly6* upregulated in V3000-infected brains and correlating with extensive brain inflammation (Gupta et al. 2017). Examination of differentially expressed genes by genome-wide microarray following infection of human skeletal muscle myoblasts with CHIKV revealed several host pathways involved in innate immune responses, cell growth and death, and virus replication (Hussain et al. 2016). A microarray of CHIKV-infected HEK-293T cells used to examine post-transcriptional regulation of gene expression identified 152 differentially regulated miRNAs that targeted three major pathways: TGF-β, endocytosis, and the cell cycle (Saxena et al. 2013).

Next generation RNA-sequencing (RNA-Seq) has also been used to identify alterations in the mRNA transcriptome following alphavirus infection (Fig. 1c). Examination of differentially expressed genes in VEEV Trinidad donkey-infected human astrocytoma U87MG cells by RNA-Seq of poly(A) and mRNAs has identified major involved pathways including the interferon and unfolded protein response (Baer et al. 2016). RNA-Seq analysis of lymph nodes and feet of CHIKV-infected mice found a marked upregulation of type I IFN-induced ISGs, as well as granzymes A, B, and K (Wilson et al. 2017). Deep sequencing of genes from a knockout human cell library infected with a vesicular stomatitis virus pseudotype bearing CHIKV envelope proteins or CHIKV Thai#16,856 isolate identified N-sulfation of heparin sulfate as critical for CHIKV infectivity (Tanaka et al. 2017).

Genetic screens using emerging technology, such as CRISPR/Cas9, are rapidly enhancing our ability to identify host cellular and immune factors that are important in virus infection and pathogenesis. A genome-wide CRISPR/Cas9 screen targeting over 20,000 mouse genes delivered by lentiviruses identified *Mxra8*, a cell adhesion molecule expressed on multiple cell types, as a receptor for several arthritogenic alphaviruses, including CHIKV, RRV, Mayaro virus, and O'nyong nyong virus, but not encephalitic alphaviruses (Zhang et al. 2018). CHIKV was shown to bind to Mxra8 on cells, resulting in enhanced attachment and internalization, and mice administered anti-Mxra8 antibodies showed lower titers and reduced foot swelling following infection with CHIKV or O'nyong nyong virus. Increased use of these newer genetic screening technologies will continue to expand our knowledge of host proteins and receptors that play an important role in the pathogenesis of alphavirus infection and may help identify potential targets for antiviral therapies.

Candidate gene studies

While genome-wide genetic screens have identified many ways in which host genetics affect how alphaviruses replicate and induce clinical disease, most of our understanding of genetic control of alphavirus pathogenesis comes from candidate genes studies in mice (Fig. 1d). The SINV mouse model of alphavirus encephalomyelitis has been used extensively to examine the role of individual genes on immunopathogenesis, and the innate viral immune response is critical for early control of SINV replication. Pattern recognition receptor (PRR) signaling induces an antiviral state through inducing expression of type I IFNs, ISGs, and pro-inflammatory cytokines and chemokines by interferon regulatory factors (IRFs) and NF-kB translocating into the nucleus. UNC93b1-mutant mice, which lack endosomal TLR signaling due to a point mutation in an endoplasmic reticulum protein that fails to transfer TLR3, TLR7, and TLR8 to endosomes, showed accelerated mortality compared to wild-type (WT) controls but still mounted an adequate type I IFN response (Esen et al. 2012). This accelerated mortality was hypothesized to be due to impaired recruitment of leukocytes to the brain rather than due to impaired TLR response, indicating natural redundancy in the PRR system. Mice deficient in TLR3 alone or MyD88, an adaptor protein for several TLRs, did not show differences in mortality compared to WT mice, further supporting this hypothesis (Esen et al. 2012; Wollish et al. 2013). In contrast, *Trif*−/− mice, which lack the adaptor protein for TLR3, show accelerated mortality, suggesting that the TRIF may play a larger role in modulating SINV pathogenesis (Wollish et al. 2013).

However, it is well established that intact type I IFN (IFN-α and IFN-β) signaling is essential for control of early viral replication and survival during alphavirus encephalomyelitis. Mice deficient in IRF7, a transcription factor activated by PRR signaling that plays a major role in activation of IFN-α and IFN-β transcription, show 100% mortality following SINV NSV infection (Esen et al. 2012). Treatment of VEEV-infected mice with an inhibitor of IKKβ, part of the NF-κB-modulating IKK complex, leads to a reduction in mortality in mice (Amaya et al. 2014). Mice deficient in Type I IFN (*Ifnb*−/−), Type I IFN receptor (*Ifnar*−/−), or downstream Type I IFN signaling (*Stat1*−/−) show higher mortality and increased SINV replication in the CNS, particularly at early time points (Burdeinick-Kerr et al. 2007; Byrnes et al. 2000; Ryman et al. 2000; Wollish et al. 2013).

Type I IFN signaling induces transcription of hundreds of ISGs involved in the antiviral response, and many of these ISGs have been shown to play an important role in the pathogenesis of alphavirus encephalomyelitis. Overexpression of ISG15 in *Ifnar*−/−, *Isg15*−/−, or CD1 mice infected with SINV reduces mortality and decreases SINV replication without affecting viral dissemination by promoting protein conjugation between ISG15 and UbE1L (Giannakopoulos et al. 2009; Lenschow et al. 2005, 2007; Zhang et al. 2007). Peripheral infection of *Irf2*−/− mice with the neurovirulent but non-neuroinvasive SVN strain of SINV leads to viral replication in the brain, clinical encephalitis, and death, indicating that IRF2, an ISG that negatively regulates IFN signaling, protects mice from neuroinvasion through promotion of immune cell development (Li et al. 2016). Mice deficient in zinc antiviral protein (ZAP), a type I IFN-induced antiviral protein that binds viral mRNAs and inhibits virus replication, and deficient in the related but type I IFN-independent TIPARP have increased mortality compared to WT mice following SINV infection (Kozaki et al. 2015, 2017; Wang et al. 2016). Overexpression of other type I IFN-induced ISGs during SINV infection, including Viperin/RSAD2 and ISG20, reduces neonatal CD1 mouse mortality (Zhang et al. 2007). In contrast, *Ddx60*−/− mice, which lack a type I IFN-inducible RNA helicase reported to bind viral RNA and associate with RIG-I-like receptors to enhance MAVS signaling in the cytoplasm, showed no change in disease outcome following SINV infection (Goubau et al. 2015). Infection of mice that lack IFIT1, an ISG that interacts with 5′UTRs of several alphaviruses to inhibit viral translation during infection, restores neurovirulence of the attenuated TC83 strain of VEEV (Cain et al. 2017; Hyde et al 2014; Reynaud et al. 2015).

The role of the innate immune response in the pathogenesis of CHIKV and other Old World alphaviruses has also been studied. Multiple PRRs have been shown to be involved in CHIKV control in vivo. Endosomal PRRs such as TLR3 and its downstream adaptor molecule TRIF provide a protective role, as *Tlr3*−/− and *Trif*−/− mice show increased foot swelling, viremia, and tissue viral burden following CHIKV infection (Her et al. 2015; Rudd et al. 2012). MYD88, the downstream adaptor of endosomal viral-RNA-sensing TLR7 among other TLRs, inhibits viral dissemination, particularly at later time points (Schilte et al. 2010). MyD88-mediated signaling through TLR7 appears to be especially important during RRV pathogenesis, with *Tlr7*−/− and *Myd88*−/− mice each showing more severe clinical disease, impaired weight gain, and enhanced muscle damage following infection (Neighbours et al. 2012). Reduced foot swelling and increased viral replication is also seen in CHIKV-infected mice deficient in MAVS/CARDIF, though not in mice deficient in upstream RIG-I or MDA5, indicating redundancy in cytoplasmic RIG-I-like receptors in CHIKV RNA sensing (Rudd et al. 2012; Schilte et al. 2010). Viral glycan interactions with c-type lectin receptors (CLR) have been shown to modulate alphavirus infection, with CHIKV-infected DCIR−/− mice developing more severe foot swelling and joint inflammation (Long et al. 2013). The pathological changes are not due to a general

CLR mechanism, as disease course and outcome in CHIKV-infected SIGNR3−/− and CD-SIGN−/− mice was indistinguishable from WT mice (Long et al. 2013). Downstream PRR-activated transcription factors IRF3 and IRF7 appear to play a redundant role in type I IFN induction, as *Irf3*−/− and *Irf7*−/− single knockout mice all survive CHIKV infection; however, *Irf3*−/−*Irf7*−/− double knockout mice show 100% mortality and massive upregulation of pro-inflammatory cytokines TNF-α, IL-6, and CCL2 (Rudd et al. 2012; Schilte et al. 2012). Deficiency of IRF-1 results in increased foot swelling and enhanced viral dissemination due to altered local pro-inflammatory cytokine and chemokine responses in mice during CHIKV infection (Nair et al. 2017). Similar to SINV studies, *Ifnar*−/− or *Stat1*−/− mice are highly susceptible to CHIKV and show increased mortality following infection (Couderc et al. 2008; Gardner et al. 2012; Partidos et al. 2011; Schilte et al. 2010).

ISGs induced following CHIKV infection are not as well described compared to SINV. Neonatal *Isg15*−/− mice show 100% mortality to CHIKV infection, but in contrast to SINV, via a mechanism independent of UbE1L-ISG15 protein conjugates (Werneke et al. 2011). *Ifitm3*−/− mice show increased ipsilateral ankle joint swelling with higher virus titers and pro-inflammatory cytokines and chemokines early in infection (Poddar et al. 2016). Viperin is highly upregulated in CHIKV-infected monocytes, and *Rsad2*−/− mice, which are deficient in the gene encoding Viperin, show increased joint inflammation and viremia compared to WT mice (Teng et al. 2012). Bone marrow stromal antigen 2 (BST-2), an ISG that tethers virions to cell surfaces and prevent budding, reduces CHIKV dissemination in mice and promotes IFN-α, IFN-γ, and CD40L expression (Mahauad-Fernandez et al. 2014). Pre-weanling *Gadd34*−/− mice show increased mortality and more severe myocardial inflammation following CHIKV infection, indicating its importance in type I IFN induction and IL-6 production (Clavarino et al. 2012).

Inflammation has been shown to drive most of the pathological changes during arthritogenic alphavirus infection. Monocytes/macrophages in particular play a complex role in CHIKV-induced joint and muscle damage. Inhibition of MCP-1/CCL2 synthesis, which is critical for macrophage recruitment, through administration of bindarit mitigates CHIKV disease development and bone loss and reduces inflammatory infiltrates in joints and muscle (Chen et al. 2015; Rulli et al. 2011). However, *Ccl2*−/− mice actually demonstrate more severe clinical disease characterized by a reduction in monocytes/macrophages but enhanced neutrophil-mediated cartilage damage and pro-inflammatory cytokine gene expression (Poo et al. 2014). Depletion of $Ly6C^{hi}CCR2+$ monocytes in CCR2-DTR transgenic mice using diphtheria toxin leads to more severe disease following CHIKV or RRV infection, via a process independent of adaptive immunity but dependent on IRF3/IRF7 and MAVS-induced type I IFN gene expression (Haist et al. 2017). Macrophages present in RRV- or CHIKV-induced lesions show a gene expression pattern consistent with M2-like macrophages, and mice deficient for *Arg1*, a gene central to M2 macrophage skewing, in macrophages and neutrophils show improved pathology and reduced viral loads, suggesting a mechanism by which viral persistence in macrophages may occur (Stoermer et al. 2012).

During RRV infection of mice, the complement system has been shown to significantly contribute to tissue damage in joints and muscles (Morrison et al. 2007, 2008). C3−/− mice, which are deficient in the central component of the complement system, and CD11b−/− mice, which are deficient in complement receptor 3, whose ligands include the C3 cleavage fragment iC3b, develop less severe disease and show reduced tissue destruction compared to WT mice despite similar tissue tropism, viral replication in tissues, and inflammatory cell recruitment, indicating that the complement system plays a large role in the effector phase of RRV-induced disease (Morrison et al. 2007, 2008). Though recruitment of inflammatory cells was not affected, absence of C3 or CR3 did decrease expression of several pro-inflammatory genes, including genes for S100A9, S100A8, and IL-6 (Morrison et al. 2008). Activation of the complement cascade during RRV infection is dependent on the mannose-binding (MBL) pathway, as MBL−/− mice, but not mice deficient in components of the classical (C1q−/− mice) or alternative (fB−/− mice) pathways, show a similar disease course as C3−/− mice (Gunn et al. 2012). In contrast, C3−/− mice peripherally infected with the V3533 strain of VEEV show a more rapid invasion of the CNS, more severe signs of encephalitis, and delayed virus clearance from the serum, suggesting that complement is critical for peripheral virus clearance to protect against neuroinvasion during encephalitic alphavirus infection (Brooke et al. 2012).

The adaptive immune response presents a double-edged sword, inducing both protective and pathological processes during alphavirus infection. The adaptive immune response, particularly T cells, has been shown to induce most of the pathological changes during alphavirus encephalomyelitis. Inhibition of lymphocyte proliferation through treatment with a glutamine antagonist partially prevents development of clinical disease and persistent neurological sequelae in a mouse model of nonfatal alphavirus encephalomyelitis (Baxter et al. 2017; Potter et al. 2015). SCID mice, which lack both B cells and T cells, infected with the AR339 strain of SINV do not develop signs of neurological disease despite supporting high levels of virus replication in the brain (Levine et al. 1991). SINV NSV-induced clinical disease development and mortality coincide with infiltration of T cells into the brain, and when mice deficient in various components of cellular immunity, including TCRα−/−,

TCRβδ−/−, β2m−/−, TAP1−/−, and CD4−/− mice, but not CD8−/− mice, are infected with SINV NSV, the rate of mortality significantly decreases compared to WT mice (Kimura and Griffin 2000; Kulcsar et al. 2014; Rowell and Griffin 2002). The pathogenic role of T cell effector molecules has also been evaluated in the SINV mouse model. In one study, NSV-induced mortality was not altered in mice lacking several T cell effector molecules, including perforin (Pfp−/−), Fas (Fas^{lpr}), TNF-α receptor (TNFαR1−/−), IL-6, or IL-12; however, IFN-γ-deficient mice (*Ifng*−/−) showed a significantly reduced mortality rate compared to WT mice, suggesting that IFN-γ plays a role in fatal alphavirus encephalomyelitis (Rowell and Griffin 2002). However, a subsequent study with SINV NSV showed similar mortality rates between WT and *Ifng*−/− and *Ifngr1*−/− (IFN-γ receptor-deficient) mice, though mice deficient in IFN-γ signaling showed increased infiltration of perforin + cells and reduced mRNA expression of *Tnf* and *Il6* in the brain (Lee et al. 2013). Compared to WT mice, *Ifng*−/− and *Ifngr1*−/− mice show reduced weight loss and a less severe decrease in feed intake due to reduced pro-inflammatory cytokine production, particularly TNF-α, in the brain during infection with the nonfatal TE strain of SINV (Baxter and Griffin 2016). Deficiency of the regulatory cytokine IL-10 in mice during SINV NSV infection accelerates mortality and promotes an increase of pathogenic Th17 cells producing GM-CSF and granzyme B (Kulcsar et al. 2014). Infection of *Il10*−/− mice with TE12, a recombinant SINV strain of intermediate virulence, leads to increased mortality and slower virus clearance compared to WT mice and is associated with an enhanced Th1 response and delayed anti-SINV antibody in the CNS (Martin and Griffin 2017). Together these studies indicate that T cells, particularly CD4 + T cells, and their pro-inflammatory effector molecules mediate CNS damage during SINV infection.

While responsible for most of the pathology in the CNS, the adaptive immune response is also required for noncytolytic clearance of SINV from neurons. Virus clearance from the CNS is accomplished through a synergistic cooperation between IFN-γ and antibody directed towards the E2 glycoprotein of SINV (Binder and Griffin 2001; Levine et al. 1991). Following infection with the nonfatal TE strain of SINV, while WT C57BL/6 mice are able to clear infectious virus by 7–10 days post-infection, virus titers are readily detectable in brains of SCID mice (Burdeinick-Kerr et al. 2007). μMT mice, which are deficient in mature B cells, are also unable to clear infectious virus, though persistent titers remain lower than that of SCID mice, and while *Ifng*−/− and *Ifngr1*−/− mice are initially able to clear infectious virus, reactivation of virus can be detected between 14 and 28 days post-infection. μMT/*Ifng*−/− double knockout mice show virus titer trends intermediate to that of SCID and μMT single knockout mice, indicating that antibody and IFN-γ work cooperatively to clear virus (Burdeinick-Kerr et al. 2007). While *Ifng*−/− and *Ifngr1*−/− mice produce comparable amounts of IgM and IgG in the serum compared to WT mice, IgM, IgG2a, and IgG2b levels are reduced in the CNS (Baxter and Griffin 2016). *Ifng*−/− and *Ifngr1*−/− mice also have significantly fewer B cells and lower mRNA expression levels of B cell attracting chemokines, including *Cxcl9, Cxcl10*, and *Cxcl13*, compared to WT mice, suggesting cooperative virus clearance between IFN-γ and anti-SINV antibody occurs via IFN-γ promotion of antibody-secreting B cell chemotaxis into the CNS (Baxter and Griffin 2016). Germline IgM or IgG alone is sufficient for SINV clearance from the CNS, with AID−/− mice (only produce IgM) and sIgM−/− mice (secrete IgG but not IgM) showing comparable morbidity and virus clearance compared to WT mice (Nilaratanakul et al. 2018). In contrast, AID−/−sIgM−/− double knockout mice are unable to clear infectious virus from the brain and show persistently high viral RNA levels, further supporting the important role of anti-SINV antibody in virus clearance (Nilaratanakul et al. 2018). Further studies examining the exact mechanism by which anti-SINV antibody clears infectious virus from neurons in a noncytolytic manner are underway.

Compared to SINV infection, the role of the adaptive immune response in inducing pathology and promoting clearance during arthritogenic alphavirus infection has not been as heavily studied. However, particularly for CHIKV, both the immune response and clinical disease closely mimic that seen in patients with rheumatoid arthritis, an autoimmune disease (Miner et al. 2015). *Rag1*−/− or *Rag2*−/− mice, which lack both T cells and B cells, show minimal inflammation in joints and muscle but persistent viremia and high viral titers in joints following CHIKV and RRV infection, suggesting that the double-edged sword analogy of the immune response applies to arthritogenic alphavirus infection (Burrack et al. 2015; Hawman et al. 2013; Seymour et al. 2015; Teo et al. 2013). During CHIKV infection, CD4 + T cells specifically appear to play the major pathogenic role in inducing joint swelling; as compared to WT and *Cd8a*−/− mice, *Cd4*−/− mice show reduced footpad swelling and polymorphonuclear cell infiltration (Teo et al. 2013). In a study examining the pathologic role of effector proteins of NK cells and CD4 cells, *Ifng*−/− mice only showed mild reduction in arthritic disease following CHIKV infection (Wilson et al. 2017). In contrast, mice deficient in granzyme A (GrzA−/−), and to a lesser extent granzyme K (GrzK−/−), showed significantly reduced foot swelling and fewer NK cells and T cells compared to WT mice (Wilson et al. 2017). γδ T cells appear to play a protective role in CHIKV infection, as γδ T cell−/− mice show enhanced disease development with increased foot swelling and poorer weight gain with increased oxidative damage in ipsilateral foot and ankle joints compared to WT mice (Long

et al. 2015). μMT mice show persistent viremia for a year following CHIKV infection, indicating that similar to SINV, virus-specific antibody mediates clearances of CHIKV (Lum et al. 2013). Also similar to SINV studies in SCID mice, monoclonal CHIKV antibody treatment prevents establishment of persistent virus and allows tissue-specific clearance of persistently infected tissues in *Rag1*−/− mice (Hawman et al. 2013). CHIKV-infected *Cd4*−/− mice show reduced CHIKV-specific IgM and IgG levels that are able to fully neutralize virus, indicating that CD4 + T cells enhance, but are not required for, antibody-mediated clearance of CHIKV (Lum et al. 2013). RRV-infected *CD8a*−/− mice demonstrate increased viral RNA levels in muscle but not joint tissues, suggesting CD8 + T cells mediate viral RNA clearance in a tissue-specific manner, and passive transfer of T cells to *Rag1*−/− mice results in reduced viral RNA loads in muscle, suggesting that T cells are capable of clearing viral RNA independent of B cells/anti-RRV antibody (Burrack et al. 2015). Further studies are warranted to better understand the role of the adaptive immune response in virus clearance, particular whether IFN-γ and virus-specific antibody mediate clearance in a cooperative manner, as with SINV.

Emerging technologies for studying host genetic variation and alphavirus pathogenesis

Examination of alphavirus infection in various inbred mouse strains has provided clues on the power of host genetics in influencing disease susceptibility and outcome. SJL mice infected with the AR339 strain of SINV develop more severe encephalitic disease than BALB/c mice and have increased inflammation characterized by decreased *Il4* expression and increased CD4 + T cells producing IL-10 (Rowell and Griffin 1999). When infected with SINV NSV, C57BL/6 mice develop ascending paralysis with 100% mortality by 7–10 days post-infection, while BALB/c mice only develop mild disease and survive (Thach et al. 2000). Spinal cords of NSV-infected BALB/c mice have higher motor neuron survival rates with decreased IL-1β production and reduced decline in GLT-1 expression, a marker for glutamate excitotoxicity, which is shown to contribute to neuronal death in alphavirus encephalitis (Prow and Irani 2008). C57BL/6 mice, in contrast, show higher CNS virus titers with higher levels of infiltrating inflammatory cells and pro-inflammatory cytokine production (Kulcsar et al. 2015). Intranasal infection with the TC-83 strain of VEEV results in high mortality in C3H mice but 100% survival in C57BL/6 mice, with NK cells shown to mediate severe disease in C3H mice (Taylor et al. 2012). These observed phenotypic differences among mouse strains have lead the way for more advanced systems genetic approaches towards the role of host genetic variation on disease outcome, such as the Collaborative Cross (CC) (Fig. 2).

Studies of quantitative trait loci (QTL) allow for identification of genomic DNA and associated genes that correlate with variation in phenotype, providing a mechanism by which the role of host genetics in alphavirus pathogenesis may be examined. While QTL analysis represents an increasingly used technology in examining complex trait contributions to infectious disease outcome and has been examined in several RNA viruses, including as influenza virus and Theiler's murine encephalitis virus (Abbas et al. 2018; Bieber et al. 2010; Boivin et al. 2012; Butterfield et al. 2003; Ferris et al. 2013; Spach et al. 2010), to date only one study has been published examining mammalian alphavirus infection. Expanding on known phenotypic differences in disease outcome between C57BL/6 and BALB/c mice infected with SINV NSV, interval mapping of CXB recombinant inbred (RI) mice identified a QTL on chromosome 2 near marker D2Mit447, designated as *Nsv1*, that correlated with viral load, development of paralysis, and death (Thach et al. 2001). Infection susceptibility to salmonoid alphavirus (SAV), a non-mammalian alphavirus, and severity of pathological lesions has been found to vary by strain of Atlantic salmon and region during natural infection (McLoughlin et al. 2003, 2006). Experimental SAV infection of different salmon populations revealed a moderate to high heritability for host resistance to SAV-induced disease, with a disease resistance QTL mapped to chromosome 3 (Gonen et al. 2015). SNPs identified to be in linkage disequilibrium with this QTL are now being used in selective breeding programs in commercial fisheries to enhance disease resistance. Future studies identifying QTL involved in disease outcome will further our understanding in the role complex trait variation plays in alphavirus pathogenesis.

The Collaborative Cross (CC) is a genetic resource population consisting of approximately one hundred fully inbred, and therefore completely reproducible, recombinant inbred (RI) lines, with both genotypes and whole-genome sequences available (Churchill et al. 2004). The eight founder lines used to create the CC consist of five classical inbred strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, and NZO/HILtJ) and three wild-derived strains from *Mus musculus* subspecies (CAST/EiJ, PWK/PhJ, and WSB/EiJ), which capture approximately 90% of known genetic variation in laboratory mice in a randomly distributed manner across the genome and create for more genetic diversity than standard RI lines created from two inbred strains. Studies using the CC have been performed using multiple viruses, including influenza virus, Ebola virus, SARS-CoV, and West Nile virus, and show that there is significant genetic variation associated with viral infection (Ferris et al. 2013; Graham et al. 2015; Gralinski et al. 2015, 2017; Rasmussen et al. 2014). Other emerging mouse systems genetics tools include the Diversity Outbred (DO) mouse population, which was derived from

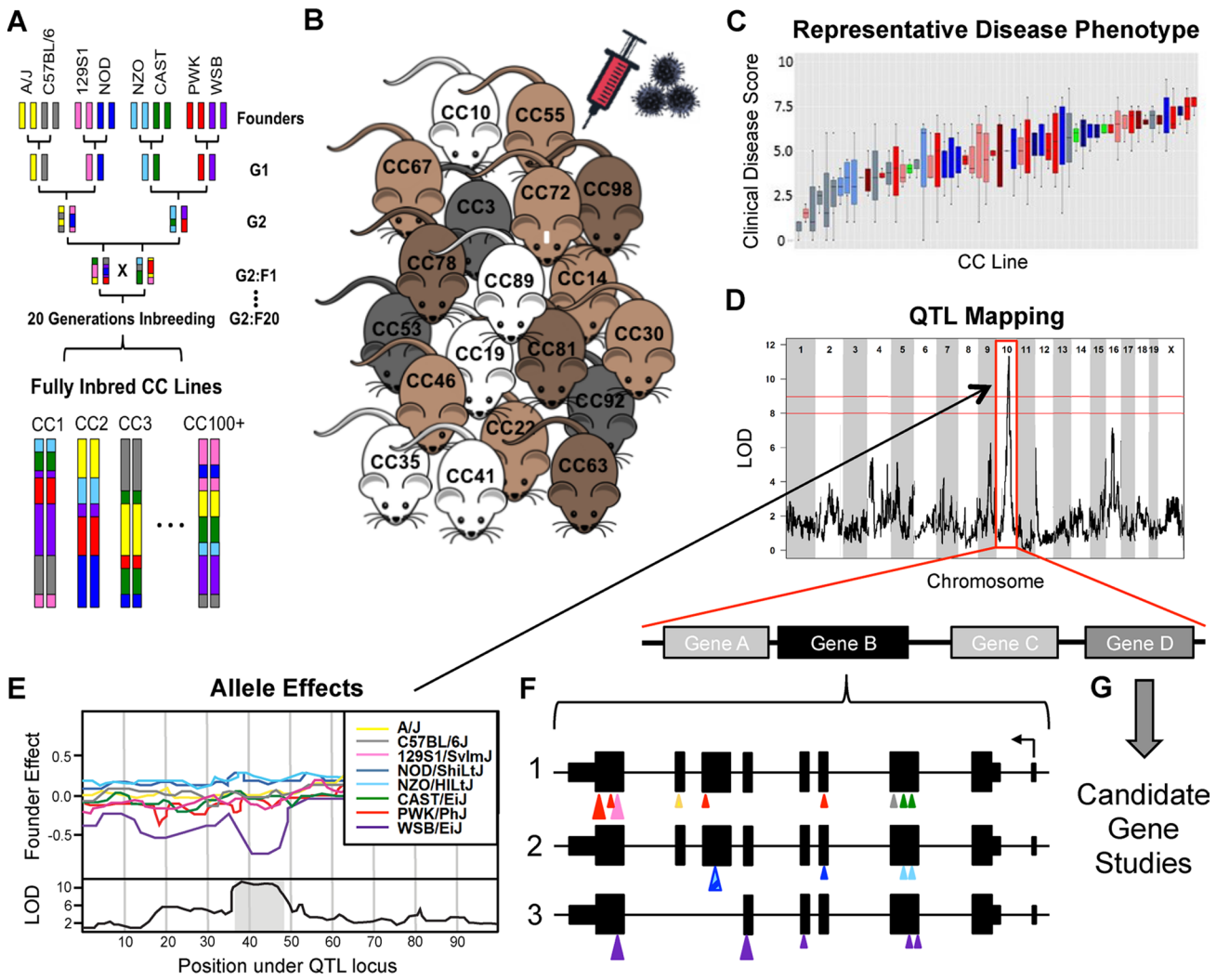

Fig. 2 Systems genetics approach to studying the role of host genetics on alphavirus pathogenesis using the Collaborative Cross (CC). **a** CC mouse populations are created by crossing eight inbred founder lines (yellow = A/J, gray = C57BL/6J, pink = 129S1/SvlmJ, dark blue = NOD/ShiLtJ, light blue = NZO/HiLtJ, green = CAST/EiJ, red = PWK/PhJ, purple = WSB/EiJ) and then inbreeding them to create a panel of fully reproducible lines. **b** In a model experiment, mice from different CC lines are infected with an alphavirus, and **c** disease phenotypes are evaluated across all infected CC lines. **d** QTL mapping is used to identify genome regions that contribute to phenotypic variation among alphavirus-infected CC lines. A QTL associated with variation in clinical disease severity is identified within chromosome 10, with the lower red line indicating $p = 0.1$ and the upper red line indicating $p = 0.05$. **e** An allele effects plot for the QTL shows that the locus is primarily driven by a WSB/EiJ founder effect. **f** Sequence data for genes within the QTL can be accessed using the Sanger Mouse Genomes Database (https://www.sanger.ac.uk/sanger/Mouse_SnpViewer/rel-1303) to identify haplotypes across the eight founder strains (Haplotype 1 = A/J, C57BL/6J, 129S1/SvlmJ, CAST/EiJ, PWK/PhJ; Haplotype 2 = NOD/ShiLtJ, NZO/HiltJ; Haplotype 3 = WSB/EiJ). Polymorphisms are denoted by arrows, with coding changes denoted by large arrows, and noncoding changes denoted by small arrows. Founder strains possessing each polymorphism are denoted by colors indicated above. **g** Candidate gene studies are performed to better understand the contribution of specific genes within the QTL to the phenotype in question. (Color figure online)

and shares the same genetic information as the CC, but is maintained using a strict randomized breeding scheme, maximizing allelic heterozygosity (Churchill et al. 2012). These emerging genetic resources permit the mapping of complex traits and allow for characterization of polymorphic genes that influence disease outcomes across diverse populations.

Summary

Completed studies examining the natural course of infection in humans and experimental infection using cell culture and animal models show we have only scratched the surface of understanding how host genetics influence

alphavirus infection. Genome-wide genetic screens and candidate gene studies provide insight to how host genes mediate or resist virus entry and replication within a cell or respond to virus infection to affect disease outcome. These studies show that the immune response in particular plays a complicated role in alphavirus infection, both mitigating and contributing to disease development and severity. While the innate immune response plays a critical role in restricting initial virus infection and replication, and the adaptive immune response mediates clearance of virus following successful infection, both arms have been shown to significantly facilitate clinical disease and tissue pathology. In addition to host genetics, alphavirus pathogenesis is further complicated by the role viral genetics play in disease development and outcome. Effective treatments for alphaviruses infection will require acknowledgement and consideration of how both viral and host genetics contribute to the pathogenesis of disease. Emerging technologies, such as systems genetics approaches using the Collaborative Cross, will further our understanding of the how host genetics contribute to alphavirus pathogenesis, and effectively harnessing this knowledge and parlaying it into successful therapies will be critical for effectively responding to new emergences and disease manifestations produced by these re-emerging and constantly evolving alphaviruses.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Abbas RMF, Torigoe D, Kameda Y et al (2018) Verification of genetic loci responsible for the resistance/susceptibility to the Sendai virus infection using congenic mice. Infect Genet Evol 57:75–81. https://doi.org/10.1016/j.meegid.2017.11.008

Adouchief S, Smura T, Sane J, Vapalahti O, Kurkela S (2016) Sindbis virus as a human pathogen—epidemiology, clinical picture and pathogenesis. Rev Med Virol 26:221–241. https://doi.org/10.1002/rmv.1876

Alla SAO, Combe B (2011) Arthritis after infection with Chikungunya virus. Best Pract Res Clin Rheumatol 25:337–346. https://doi.org/10.1016/j.berh.2011.03.005

Amaya M, Voss K, Sampey G et al (2014) The role of IKKβ in Venezuelan equine encephalitis virus infection. PLoS ONE 9:e86745. https://doi.org/10.1371/journal.pone.0086745

Anfasa F, Provacia L, GeurtsvanKessel C et al (2017) Hyperferritinemia is a potential marker of chronic chikungunya: a retrospective study on the Island of Curaçao during the 2014–2015 outbreak. J Clin Virol 86:31–38. https://doi.org/10.1016/j.jcv.2016.11.003

Aunsmo A, Valle PS, Sandberg M et al (2010) Stochastic modelling of direct costs of pancreas disease (PD) in Norwegian farmed Atlantic salmon (*Salmo salar* L.). Prev Vet Med 93:233–241. https://doi.org/10.1016/j.prevetmed.2009.10.001

Badawi A, Ryoo SG, Vasileva D, Yaghoubi S (2018) Prevalence of chronic comorbidities in chikungunya: a systematic review and meta-analysis. Int J Infect Dis 67:107–113. https://doi.org/10.1016/j.ijid.2017.12.018

Baer A, Lundberg L, Swales D et al (2016) Venezuelan equine encephalitis virus induces apoptosis through the unfolded protein response activation of EGR1. J Virol 90:3558–3572. https://doi.org/10.1128/JVI.02827-15

Baxter VK, Griffin DE (2016) Interferon gamma modulation of disease manifestation and the local antibody response to alphavirus encephalomyelitis. J Gen Virol 97:2908–2925. https://doi.org/10.1099/jgv.0.000613

Baxter VK, Glowinski R, Braxton AM et al (2017) Glutamine antagonist-mediated immune suppression decreases pathology but delays virus clearance in mice during nonfatal alphavirus encephalomyelitis. Virology 508:134–149. https://doi.org/10.1016/j.virol.2017.05.013

Bieber AJ, Suwansrinon K, Kerkvliet J et al (2010) Allelic variation in the Tyk2 and EGF genes as potential genetic determinants of CNS repair. Proc Natl Acad Sci USA 107:792–797. https://doi.org/10.1073/pnas.0906589107

Binder GK, Griffin DE (2001) Interferon-gamma-mediated site-specific clearance of alphavirus from CNS neurons. Science 293:303–306. https://doi.org/10.1126/science.1059742

Boivin GA, Pothlichet J, Skamene E et al (2012) Mapping of clinical and expression quantitative trait loci in a sex-dependent effect of host susceptibility to mouse-adapted influenza H3N2/HK/1/68. J Immunol 188:3949–3960. https://doi.org/10.4049/jimmunol.1103320

Borgherini G, Poubeau P, Jossaume A et al (2008) Persistent arthralgia associated with chikungunya virus: a study of 88 adult patients on reunion island. Clin Infect Dis 47:469–475. https://doi.org/10.1086/590003

Brooke CB, Schäfer A, Matsushima GK et al (2012) Early activation of the host complement system is required to restrict central nervous system invasion and limit neuropathology during Venezuelan equine encephalitis virus infection. J Gen Virol 93:797–806. https://doi.org/10.1099/vir.0.038281-0

Bruyn HB, Lennette EH (1953) Western equine encephalitis in infants; a report on three cases with sequelae. Calif Med 79:362–366

Burdeinick-Kerr R, Wind J, Griffin DE (2007) Synergistic roles of antibody and interferon in noncytolytic clearance of Sindbis virus from different regions of the central nervous system. J Virol 81:5628–5636. https://doi.org/10.1128/JVI.01152-06

Burrack KS, Montgomery SA, Homann D, Morrison TE (2015) CD8 + T cells control Ross River virus infection in musculoskeletal tissues of infected mice. J Immunol 194:678–689. https://doi.org/10.4049/jimmunol.1401833

Butterfield RJ, Roper RJ, Rhein DM et al (2003) Sex-specific quantitative trait loci govern susceptibility to Theiler's murine encephalomyelitis virus-induced demyelination. Genetics 163:1041–1046

Byrnes AP, Durbin JE, Griffin DE (2000) Control of Sindbis virus infection by antibody in interferon-deficient mice. J Virol 74:3905–3908

Cain MD, Salimi H, Gong Y et al (2017) Virus entry and replication in the brain precedes blood-brain barrier disruption during intranasal alphavirus infection. J Neuroimmunol 308:118–130. https://doi.org/10.1016/j.jneuroim.2017.04.008

Calisher CH (1994) Medically important arboviruses of the United States and Canada. Clin Microbiol Rev 7:89–116

Cerny T, Schwarz M, Schwarz U et al (2017) The range of neurological complications in chikungunya fever. Neurocrit Care 27:447–457. https://doi.org/10.1007/s12028-017-0413-8

Chaaitanya IK, Muruganandam N, Sundaram SG et al (2011) Role of proinflammatory cytokines and chemokines in chronic arthropathy in CHIKV infection. Viral Immunol 24:265–271. https://doi.org/10.1089/vim.2010.0123

Chaaithanya IK, Muruganandam N, Anwesh M et al (2013) HLA class II allele polymorphism in an outbreak of chikungunya fever in Middle Andaman, India. Immunology 140:202–210. https://doi.org/10.1111/imm.12128

Chang AY, Encinales L, Porras A et al (2017) Frequency of chronic joint pain following chikungunya infection: a Colombian cohort study. Arthritis Rheumatol. https://doi.org/10.1002/art.40384

Charles PC, Walters E, Margolis F, Johnston RE (1995) Mechanism of neuroinvasion of Venezuelan equine encephalitis virus in the mouse. Virology 208:662–671. https://doi.org/10.1006/viro.1995.1197

Chen W, Foo S-S, Taylor A et al (2015) Bindarit, an inhibitor of monocyte chemotactic protein synthesis, protects against bone loss induced by chikungunya virus infection. J Virol 89:581–593. https://doi.org/10.1128/JVI.02034-14

Chirathaworn C, Rianthavorn P, Wuttirattanakowit N, Poovorawan Y (2010) Serum IL-18 and IL-18BP levels in patients with Chikungunya virus infection. Viral Immunol 23:113–117. https://doi.org/10.1089/vim.2009.0077

Chirathaworn C, Poovorawan Y, Lertmaharit S, Wuttirattanakowit N (2013) Cytokine levels in patients with chikungunya virus infection. Asian Pac J Trop Med 6:631–634. https://doi.org/10.1016/S1995-7645(13)60108-X

Chopra A, Saluja M, Venugopalan A (2014) Effectiveness of chloroquine and inflammatory cytokine response in patients with early persistent musculoskeletal pain and arthritis following chikungunya virus infection. Arthritis Rheumatol 66:319–326. https://doi.org/10.1002/art.38221

Chow A, Her Z, Ong EKS et al (2011) Persistent arthralgia induced by Chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor. J Infect Dis 203:149–157. https://doi.org/10.1093/infdis/jiq042

Churchill GA, Airey DC, Allayee H et al (2004) The collaborative cross, a community resource for the genetic analysis of complex traits. Nat Genet 36:1133–1137. https://doi.org/10.1038/ng1104-1133

Churchill GA, Gatti DM, Munger SC, Svenson KL (2012) The diversity outbred mouse population. Mamm Genome 23:713–718. https://doi.org/10.1007/s00335-012-9414-2

Clavarino G, Cláudio N, Couderc T et al (2012) Induction of GADD34 is necessary for dsRNA-dependent interferon-β production and participates in the control of Chikungunya virus infection. PLoS Pathog 8:e1002708. https://doi.org/10.1371/journal.ppat.1002708

Couderc T, Chrétien F, Schilte C et al (2008) A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. PLoS Pathog 4:e29. https://doi.org/10.1371/journal.ppat.0040029

Cvejic E, Lemon J, Hickie IB et al (2014) Neurocognitive disturbances associated with acute infectious mononucleosis, Ross River fever and Q fever: a preliminary investigation of inflammatory and genetic correlates. Brain Behav Immun 36:207–214. https://doi.org/10.1016/j.bbi.2013.11.002

Dendrou CA, Petersen J, Rossjohn J, Fugger L (2018) HLA variation and disease. Nat Rev Immunol 8:193. https://doi.org/10.1038/nri.2017.143

DeTulleo L, Kirchhausen T (1998) The clathrin endocytic pathway in viral infection. EMBO J 17:4585–4593

Dubuisson J, Lustig S, Ruggli N, Akov Y, Rice CM (1997) Genetic determinants of Sindbis virus neuroinvasiveness. J Virol 71:2636–2646

Dutta SK, Tripathi A (2017) Association of toll-like receptor polymorphisms with susceptibility to chikungunya virus infection. Virology 511:207–213. https://doi.org/10.1016/j.virol.2017.08.009

Elsinga J, Gerstenbluth I, van der Ploeg S et al (2017) Long-term chikungunya sequelae in Curaçao: burden, determinants, and a novel classification tool. J Infect Dis 216:573–581. https://doi.org/10.1093/infdis/jix312

Erwin-Cohen RA, Porter AI, Pittman PR et al (2017) Human transcriptome response to immunization with live-attenuated Venezuelan equine encephalitis virus vaccine (TC-83): analysis of whole blood. Hum Vaccin Immunother 13:169–179. https://doi.org/10.1080/21645515.2016.1227900

Esen N, Blakely PK, Rainey-Barger EK, Irani DN (2012) Complexity of the microglial activation pathways that drive innate host responses during lethal alphavirus encephalitis in mice. ASN Neuro 4:207–221. https://doi.org/10.1042/AN20120016

Ferris MT, Aylor DL, Bottomly D et al (2013) Modeling host genetic regulation of influenza pathogenesis in the collaborative cross. PLoS Pathog 9:e1003196–e1003115. https://doi.org/10.1371/journal.ppat.1003196

Fraser JR, Cunningham AL, Clarris BJ et al (1981) Cytology of synovial effusions in epidemic polyarthritis. Aust N Z J Med 11:168–173

Gallian P, Leparc-Goffart I, Richard P et al (2017) Epidemiology of chikungunya virus outbreaks in Guadeloupe and Martinique, 2014: an observational study in volunteer blood donors. PLoS Negl Trop Dis 11:e0005254. https://doi.org/10.1371/journal.pntd.0005254

Ganesan K, Diwan A, Shankar SK et al (2008) Chikungunya encephalomyeloradiculitis: report of 2 cases with neuroimaging and 1 case with autopsy findings. Am J Neuroradiol 29:1636–1637. https://doi.org/10.3174/ajnr.A1133

Gardner CL, Burke CW, Higgs ST et al (2012) Interferon-alpha/beta deficiency greatly exacerbates arthritogenic disease in mice infected with wild-type chikungunya virus but not with the cell culture-adapted live-attenuated 181/25 vaccine candidate. Virology 425:103–112. https://doi.org/10.1016/j.virol.2011.12.020

Gauri LA, Thaned A, Fatima Q et al (2016) Clinical spectrum of chikungunya in Bikaner (North Western India) in 2006 and follow up of patients for five years. J Assoc Physicians India 64:22–25

Gérardin P, Fianu A, Malvy D et al (2011) Perceived morbidity and community burden after a Chikungunya outbreak: the TELECHIK survey, a population-based cohort study. BMC Med 9:5. https://doi.org/10.1186/1741-7015-9-5

Giannakopoulos NV, Arutyunova E, Lai C et al (2009) ISG15 Arg151 and the ISG15-conjugating enzyme UbE1L are important for innate immune control of Sindbis virus. J Virol 83:1602–1610. https://doi.org/10.1128/JVI.01590-08

Go YY, Balasuriya UBR, Lee C-K (2014) Zoonotic encephalitides caused by arboviruses: transmission and epidemiology of alphaviruses and flaviviruses. Clin Exp Vaccine Res 3:58–20. https://doi.org/10.7774/cevr.2014.3.1.58

Goldfield M, Welsh JN, Taylor BF (1968) The 1959 outbreak of Eastern Encephalitis in New Jersey. 4. CF reactivity following overt and inapparent infection. Am J Epidemiol 87:23–31

Gonen S, Baranski M, Thorland I et al (2015) Mapping and validation of a major QTL affecting resistance to pancreas disease (salmonid alphavirus) in Atlantic salmon (Salmo salar). Heredity 115:405–414. https://doi.org/10.1038/hdy.2015.37

Goubau D, van der Veen AG, Chakravarty P et al (2015) Mouse superkiller-2-like helicase DDX60 is dispensable for type I IFN induction and immunity to multiple viruses. Eur J Immunol 45:3386–3403. https://doi.org/10.1002/eji.201545794

Graham JB, Thomas S, Swarts J et al (2015) Genetic diversity in the collaborative cross model recapitulates human West Nile virus disease outcomes. mBio 6:e00493–e00415. https://doi.org/10.1128/mBio.00493-15

Gralinski LE, Ferris MT, Aylor DL et al (2015) Genome wide identification of SARS-CoV susceptibility loci using the collaborative cross. PLoS Genet 11:e1005504–e1005521. https://doi.org/10.1371/journal.pgen.1005504

Gralinski LE, Menachery VD, Morgan AP et al (2017) Allelic variation in the toll-like receptor adaptor protein Ticam2 contributes to SARS-coronavirus pathogenesis in mice. G3 (Bethesda) 7:1653–1663. https://doi.org/10.1534/g3.117.041434

Greenlee JE (2014) The equine encephalitides. Handb Clin Neurol 123:417–432. https://doi.org/10.1016/B978-0-444-53488-0.00019-5

Griffin DE (2010) Emergence and re-emergence of viral diseases of the central nervous system. Prog Neurobiol 91:95–101. https://doi.org/10.1016/j.pneurobio.2009.12.003

Griffin DE (2013) Alphaviruses. In: Knipe DM, Howley PM (eds) Fields virology, 6 edn. Lippincott Williams & Wilkins, Philadelphia, pp 652–686

Griffin DE, Levine B, Tyor WR, Irani DN (1992) The immune response in viral encephalitis. Semin Immunol 4:111–119

Gunn BM, Morrison TE, Whitmore AC et al (2012) Mannose binding lectin is required for alphavirus-induced arthritis/myositis. PLoS Pathog 8:e1002586. https://doi.org/10.1371/journal.ppat.1002586

Gupta P, Sharma A, Han J et al (2017) Differential host gene responses from infection with neurovirulent and partially-neurovirulent strains of Venezuelan equine encephalitis virus. BMC Infect Dis 17:309. https://doi.org/10.1186/s12879-017-2355-3

Haist KC, Burrack KS, Davenport BJ, Morrison TE (2017) Inflammatory monocytes mediate control of acute alphavirus infection in mice. PLoS Pathog 13:e1006748. https://doi.org/10.1371/journal.ppat.1006748

Hatanpaa KJ, Kim JH (2014) Neuropathology of viral infections. Handb Clin Neurol 123:193–214. https://doi.org/10.1016/B978-0-444-53488-0.00008-0

Hawman DW, Stoermer KA, Montgomery SA et al (2013) Chronic joint disease caused by persistent chikungunya virus infection is controlled by the adaptive immune response. J Virol 87:13878–13888. https://doi.org/10.1128/JVI.02666-13

Heath CJ, Lowther J, Noël TP et al (2018) The identification of risk factors for chronic chikungunya arthralgia in Grenada, West Indies: a cross-sectional cohort study. Open Forum Infect Dis 5:ofx234. https://doi.org/10.1093/ofid/ofx234

Her Z, Teng T-S, Tan JJL et al (2015) Loss of TLR3 aggravates CHIKV replication and pathology due to an altered virus-specific neutralizing antibody response. EMBO Mol Med 7:24–41. https://doi.org/10.15252/emmm.201404459

Hoarau JJ, Jaffar-Bandjee MC, Krejbich-Trotot P et al (2010) Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. J Immunol 184:5914–5927. https://doi.org/10.4049/jimmunol.0900255

Hollidge BS, González-Scarano F, Soldan SS (2010) Arboviral encephalitides: transmission, emergence, and pathogenesis. J Neuroimmune Pharmacol 5:428–442. https://doi.org/10.1007/s11481-010-9234-7

Honnold SP, Mossel EC, Bakken RR et al (2015) Eastern equine encephalitis virus in mice II: pathogenesis is dependent on route of exposure. Virol J 12:154. https://doi.org/10.1186/s12985-015-0385-2

Huang Y, Paxton WA, Wolinsky SM et al (1996) The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat Med 2:1240–1243

Hussain KM, Lee RCH, Ng MM-L, Chu JJH (2016) Establishment of a novel primary human skeletal myoblast cellular model for chikungunya virus infection and pathogenesis. Sci Rep 6:21406. https://doi.org/10.1038/srep21406

Hyde JL, Gardner CL, Kimura T et al (2014) A viral RNA structural element alters host recognition of non-self RNA. Science 343:783–787. https://doi.org/10.1126/science.1248465

Janssen R, Bont L, Siezen CLE et al (2007) Genetic susceptibility to respiratory syncytial virus bronbhiolitis is predominantly associated with innate immune genes. J Infect Dis 196:826–834. https://doi.org/10.1086/520886

Kam Y-W, Simarmata D, Chow A et al (2012) Early appearance of neutralizing immunoglobulin G3 antibodies is associated with chikungunya virus clearance and long-term clinical protection. J Infect Dis 205:1147–1154. https://doi.org/10.1093/infdis/jis033

Karlas A, Berre S, Couderc T et al (2016) A human genome-wide loss-of-function screen identifies effective chikungunya antiviral drugs. Nat Commun 7:11320. https://doi.org/10.1038/ncomms11320

Kashyap RS, Morey S, Bhullar S et al (2014) Determination of toll-like receptor-induced cytokine profiles in the blood and cerebrospinal fluid of Chikungunya patients. Neuroimmunomodulation 21:338–346. https://doi.org/10.1159/000358240

Kelvin AA, Banner D, Silvi G et al (2011) Inflammatory cytokine expression is associated with chikungunya virus resolution and symptom severity. PLoS Negl Trop Dis 5:e1279. https://doi.org/10.1371/journal.pntd.0001279

Kimura T, Griffin DE (2000) The role of CD8(+) T cells and major histocompatibility complex class I expression in the central nervous system of mice infected with neurovirulent Sindbis virus. J Virol 74:6117–6125

Kimura T, Griffin DE (2003) Extensive immune-mediated hippocampal damage in mice surviving infection with neuroadapted Sindbis virus. Virology 311:28–39. https://doi.org/10.1016/S0042-6822(03)00110-7

Kozaki T, Takahama M, Misawa T et al (2015) Role of zinc-finger antiviral protein in host defense against Sindbis virus. Int Immunol 27:357–364. https://doi.org/10.1093/intimm/dxv010

Kozaki T, Komano J, Kanbayashi D et al (2017) Mitochondrial damage elicits a TCDD-inducible poly(ADP-ribose) polymerase-mediated antiviral response. Proc Natl Acad Sci USA 114:2681–2686. https://doi.org/10.1073/pnas.1621508114

Kuhn RT (2007) Togaviridae: the viruses and their replication. In: Knipe DM, Howley PM (eds) Fields virology, 5 edn. Lippincott Williams & Wilkins, Philadelphia, pp 629–650

Kulcsar KA, Baxter VK, Greene IP, Griffin DE (2014) Interleukin 10 modulation of pathogenic Th17 cells during fatal alphavirus encephalomyelitis. Proc Natl Acad Sci USA 111:16053–16058. https://doi.org/10.1073/pnas.1418966111

Kulcsar KA, Baxter VK, Abraham R et al (2015) Distinct immune responses in resistant and susceptible strains of mice during neurovirulent alphavirus encephalomyelitis. J Virol 89:8280–8291. https://doi.org/10.1128/JVI.00173-15

Labadie K, Larcher T, Joubert C et al (2010) Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J Clin Invest 120:894–906. https://doi.org/10.1172/JCI40104

Le Pendu J, Ruvoen-Clouet N, Kindberg E, Svensson L (2006) Mendelian resistance to human norovirus infections. Sem Immunol 18:375–386. https://doi.org/10.1016/j.smim.2006.07.009

Lee E-Y, Schultz KLW, Griffin DE (2013) Mice Deficient in interferon-gamma or interferon-gamma receptor 1 have distinct inflammatory responses to acute viral encephalomyelitis. PLoS ONE 8:e76412. https://doi.org/10.1371/journal.pone.0076412.g010

Lenschow DJ, Giannakopoulos NV, Gunn LJ et al (2005) Identification of interferon-stimulated gene 15 as an antiviral molecule during

Sindbis virus infection in vivo. J Virol 79:13974–13983. https://doi.org/10.1128/JVI.79.22.13974-13983.2005
Lenschow DJ, Lai C, Frias-Staheli N et al (2007) IFN-stimulated gene 15 functions as a critical antiviral molecule against influenza, herpes, and Sindbis viruses. Proc Natl Acad Sci USA 104:1371–1376. https://doi.org/10.1073/pnas.0607038104
Levine B, Hardwick JM, Trapp BD et al (1991) Antibody-mediated clearance of alphavirus infection from neurons. Science 254:856–860
Li MMH, Bozzacco L, Hoffmann H-H et al (2016) Interferon regulatory factor 2 protects mice from lethal viral neuroinvasion. J Exp Med 213:2931–2947. https://doi.org/10.1084/jem.20160303
Lohachanakul J, Phuklia W, Thannagith M et al (2012) High concentrations of circulating interleukin-6 and monocyte chemotactic protein-1 with low concentrations of interleukin-8 were associated with severe chikungunya fever during the 2009–2010 outbreak in Thailand. Microbiol Immunol 56:134–138. https://doi.org/10.1111/j.1348-0421.2011.00417.x
Long KM, Whitmore AC, Ferris MT et al (2013) Dendritic cell immunoreceptor regulates Chikungunya virus pathogenesis in mice. J Virol 87:5697–5706. https://doi.org/10.1128/JVI.01611-12
Long KM, Ferris MT, Whitmore AC et al (2015) γδ T Cells play a protective role in chikungunya virus-induced disease. J Virol 90:433–443. https://doi.org/10.1128/JVI.02159-15
Lum F-M, Teo T-H, Lee WWL et al (2013) An essential role of antibodies in the control of Chikungunya virus infection. J Immunol 190:6295–6302. https://doi.org/10.4049/jimmunol.1300304
Mahauad-Fernandez WD, Jones PH, Okeoma CM (2014) Critical role for bone marrow stromal antigen 2 in acute Chikungunya virus infection. J Gen Virol 95:2450–2461. https://doi.org/10.1099/vir.0.068643-0
Martin NM, Griffin DE (2017) IL-10 modulation of virus clearance and disease in mice with alphaviral encephalomyelitis. J Virol 92:e01517–e01517. https://doi.org/10.1128/JVI.01517-17
McLoughlin MF, Graham DA (2007) Alphavirus infections in salmonids–a review. J Fish Dis 30:511–531. https://doi.org/10.1111/j.1365-2761.2007.00848.x
McLoughlin MF, Peeler E, Foyle KL, O'Ceallachain D (2003) An epidemiological investigation of the re-emergence of pancreas disease in Irish farmed Atlantic salmon (*Salmo salar* L.) in 2002. Mar Environ Health Ser
McLoughlin MF, Graham DA, Norris A et al (2006) Virological, serological and histopathological evaluation of fish strain susceptibility to experimental infection with salmonid alphavirus. Dis Aquat Org 72:125–133. https://doi.org/10.3354/dao072125
Miner JJ, Aw Yeang HX, Fox JM et al (2015) Chikungunya viral arthritis in the United States: a mimic of seronegative rheumatoid arthritis. Arthritis Rheumatol 67:1214–1220. https://doi.org/10.1002/art.39027
Morrison TE, Whitmore AC, Shabman RS et al (2006) Characterization of Ross River virus tropism and virus-induced inflammation in a mouse model of viral arthritis and myositis. J Virol 80:737–749. https://doi.org/10.1128/JVI.80.2.737-749.2006
Morrison TE, Fraser RJ, Smith PN et al (2007) Complement contributes to inflammatory tissue destruction in a mouse model of Ross River virus-induced disease. J Virol 81:5132–5143. https://doi.org/10.1128/JVI.02799-06
Morrison TE, Simmons JD, Heise MT (2008) Complement receptor 3 promotes severe ross river virus-induced disease. J Virol 82:11263–11272. https://doi.org/10.1128/JVI.01352-08
Morrison TE, Oko L, Montgomery SA et al (2011) A mouse model of chikungunya virus-induced musculoskeletal inflammatory disease: evidence of arthritis, tenosynovitis, myositis, and persistence. Am J Pathol 178:32–40. https://doi.org/10.1016/j.ajpath.2010.11.018
Muelas N, Marti P, Azorin C et al (2017) Inflammatory myopathy related to Chikungunya virus: a case report. Neuromuscul Disord 27:S159
Nair S, Poddar S, Shimak RM, Diamond MS (2017) Interferon regulatory factor-1 (IRF-1) protects against chikungunya virus induced immunopathology by restricting infection in muscle cells. J Virol 91:e01419–e01417. https://doi.org/10.1128/JVI.01419-17
Neighbours LM, Long K, Whitmore AC, Heise MT (2012) Myd88-dependent toll-like receptor 7 signaling mediates protection from severe Ross River virus-induced disease in mice. J Virol 86:10675–10685. https://doi.org/10.1128/JVI.00601-12
Ng LFP, Chow A, Sun Y-J et al (2009) IL-1beta, IL-6, and RANTES as biomarkers of Chikungunya severity. PLoS ONE 4:e4261. https://doi.org/10.1371/journal.pone.0004261
Nilaratanakul V, Chen J, Tran O et al (2018) Germline IgM is sufficient, but not required, for antibody-mediated alphavirus clearance from the central nervous system. J Virol 92:e02081–e02017. https://doi.org/10.1128/JVI.02081-17
Ooi YS, Stiles KM, Liu CY et al (2013) Genome-wide RNAi screen identifies novel host proteins required for alphavirus entry. PLoS Pathog 9:e1003835. https://doi.org/10.1371/journal.ppat.1003835
Orvedahl A, Sumpter R, Xiao G et al (2011) Image-based genome-wide siRNA screen identifies selective autophagy factors. Nature 480:113–117. https://doi.org/10.1038/nature10546
Ozden S, Huerre M, Riviere J-P et al (2017) Human muscle satellite cells as targets of chikungunya virus infection. PLoS ONE 2:e527. https://doi.org/10.1371/journal.pone.0000527
Palmer RJ, Finley KH (1956) Sequelae of encephalitis; report of a study after the California epidemic. Calif Med 84:98–100
Panda D, Rose PP, Hanna SL et al (2013) Genome-wide RNAi screen identifies SEC61A and VCP as conserved regulators of Sindbis virus entry. Cell Rep 5:1737–1748. https://doi.org/10.1016/j.celrep.2013.11.028
Panda D, Gold B, Tartell MA et al (2015) The transcription factor FoxK participates with Nup98 to regulate antiviral gene expression. mBio 6:e02509–e02514. https://doi.org/10.1128/mBio.02509-14
Partidos CD, Weger J, Brewoo J et al (2011) Probing the attenuation and protective efficacy of a candidate chikungunya virus vaccine in mice with compromised interferon (IFN) signaling. Vaccine 29:3067–3073. https://doi.org/10.1016/j.vaccine.2011.01.076
Passoni G, Langevin C, Palha N et al (2017) Imaging of viral neuroinvasion in the zebrafish reveals that Sindbis and chikungunya viruses favour different entry routes. Dis Model Mech 10:847–857. https://doi.org/10.1242/dmm.029231
Phillips AT, Rico AB, Stauft CB et al (2016) Entry sites of Venezuelan and Western equine encephalitis viruses in the mouse central nervous system following peripheral infection. J Virol 90:5785–5796. https://doi.org/10.1128/JVI.03219-15
Poddar S, Hyde JL, Gorman MJ et al (2016) The interferon-stimulated gene IFITM3 restricts infection and pathogenesis of arthritogenic and encephalitic alphaviruses. J Virol 90:8780–8794. https://doi.org/10.1128/JVI.00655-16
Poo YS, Nakaya H, Gardner J et al (2014) CCR2 deficiency promotes exacerbated chronic erosive neutrophil-dominated chikungunya virus arthritis. J Virol 88:6862–6872. https://doi.org/10.1128/JVI.03364-13
Potter MC, Baxter VK, Mathey RW et al (2015) Neurological sequelae induced by alphavirus infection of the CNS are attenuated by treatment with the glutamine antagonist 6-diazo-5-oxo-l-norleucine. J Neurovirol 21:159–173. https://doi.org/10.1007/s13365-015-0314-6
Prow NA, Irani DN (2008) The inflammatory cytokine, interleukin-1 beta, mediates loss of astroglial glutamate transport and drives excitotoxic motor neuron injury in the spinal cord during acute

viral encephalomyelitis. J Neurochem 105:1276–1286. https://doi.org/10.1111/j.1471-4159.2008.05230.x
Radoshitzky SR, Pegoraro G, Chī XO et al (2016) siRNA screen identifies trafficking host factors that modulate alphavirus infection. PLoS Pathog 12:e1005466. https://doi.org/10.1371/journal.ppat.1005466
Rasmussen AL, Okumura A, Ferris MT et al (2014) Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance. Science 346:987–991. https://doi.org/10.1126/science.1259595
Reddy V, Mani RS, Desai A, Ravi V (2014) Correlation of plasma viral loads and presence of Chikungunya IgM antibodies with cytokine/chemokine levels during acute Chikungunya virus infection. J Med Virol 86:1393–1401. https://doi.org/10.1002/jmv.23875
Reynaud JM, Kim DY, Atasheva S et al (2015) IFIT1 differentially interferes with translation and replication of alphavirus genomes and promotes induction of type I interferon. PLoS Pathog 11:e1004863. https://doi.org/10.1371/journal.ppat.1004863
Rose PP, Hanna SL, Spiridigliozzi A et al (2011) Natural resistance-associated macrophage protein is a cellular receptor for sindbis virus in both insect and mammalian hosts. Cell HostMicrobe 10:97–104. https://doi.org/10.1016/j.chom.2011.06.009
Rowell JFJ, Griffin DED (1999) The inflammatory response to nonfatal Sindbis virus infection of the nervous system is more severe in SJL than in BALB/c mice and is associated with low levels of IL-4 mRNA and high levels of IL-10-producing CD4 + T cells. J Immunol 162:1624–1632
Rowell JF, Griffin DE (2002) Contribution of T cells to mortality in neurovirulent Sindbis virus encephalomyelitis. J Neuroimmunol 127:106–114
Rudd PA, Wilson J, Gardner J et al (2012) Interferon response factors 3 and 7 protect against Chikungunya virus hemorrhagic fever and shock. J Virol 86:9888–9898. https://doi.org/10.1128/JVI.00956-12
Rulli NE, Melton J, Wilmes A et al (2007) The molecular and cellular aspects of arthritis due to alphavirus infections: lesson learned from Ross River virus. Ann N Y Acad Sci 1102:96–108. https://doi.org/10.1196/annals.1408.007
Rulli NE, Rolph MS, Srikiatkhachorn A et al (2011) Protection from arthritis and myositis in a mouse model of acute chikungunya virus disease by bindarit, an inhibitor of monocyte chemotactic protein-1 synthesis. J Infect Dis 204:1026–1030. https://doi.org/10.1093/infdis/jir470
Ryman KD, Klimstra WB (2008) Host responses to alphavirus infection. Immunol Rev 225:27–45. https://doi.org/10.1111/j.1600-065X.2008.00670.x
Ryman KD, Klimstra WB, Nguyen KB, Biron CA (2000) Alpha/beta interferon protects adult mice from fatal Sindbis virus infection and is an important determinant of cell and tissue tropism. J Virol 74:3366–3378
Sane J, Kurkela S, Desdouits M et al (2012) Prolonged myalgia in Sindbis virus infection: case description and in vitro infection of myotubes and myoblasts. J Infect Dis 206:407–414. https://doi.org/10.1093/infdis/jis358
Saxena T, Tandon B, Sharma S et al (2013) Combined miRNA and mRNA signature identifies key molecular players and pathways involved in chikungunya virus infection in human cells. PLoS ONE 8:e79886. https://doi.org/10.1371/journal.pone.0079886
Schäfer A, Brooke CB, Whitmore AC, Johnston RE (2011) The role of the blood-brain barrier during Venezuelan equine encephalitis virus infection. J Virol 85:10682–10690. https://doi.org/10.1128/JVI.05032-11
Schilte C, Couderc T, Chrétien F et al (2010) Type I IFN controls chikungunya virus via its action on nonhematopoietic cells. J Exp Med 207:429–442. https://doi.org/10.1084/jem.20090851
Schilte C, Buckwalter MR, Laird ME et al (2012) Cutting edge: independent roles for IRF-3 and IRF-7 in hematopoietic and nonhematopoietic cells during host response to Chikungunya infection. J Immunol 188:2967–2971. https://doi.org/10.4049/jimmunol.1103185
Sepúlveda-Delgado J, Vera-Lastra OL, Trujillo-Murillo K et al (2017) Inflammatory biomarkers, disease activity index, and self-reported disability may be predictors of chronic arthritis after chikungunya infection: brief report. Clin Rheumatol 36:695–699. https://doi.org/10.1007/s10067-016-3419-2
Seymour RL, Adams AP, Leal G et al (2015) A rodent model of chikungunya virus infection in RAG1-/- mice, with features of persistence, for vaccine safety evaluation. PLoS Negl Trop Dis 9:e0003800. https://doi.org/10.1371/journal.pntd.0003800
Sharma A, Bhomia M, Honnold SP, Maheshwari RK (2011) Role of adhesion molecules and inflammation in Venezuelan equine encephalitis virus infected mouse brain. Virol J 8:197. https://doi.org/10.1186/1743-422X-8-197
Sissoko D, Malvy D, Ezzedine K et al (2009) Post-epidemic Chikungunya disease on Reunion Island: course of rheumatic manifestations and associated factors over a 15-month period. PLoS Negl Trop Dis 3:e389. https://doi.org/10.1371/journal.pntd.0000389
Spach KM, Case LK, Noubade R et al (2010) Multiple linked quantitative trait loci within the Tmevd2/Eae3 interval control the severity of experimental allergic encephalomyelitis in DBA/2J mice. Genes Immun 11:649–659. https://doi.org/10.1038/gene.2010.40
Staples JE, Breiman RF, Powers AM (2009) Chikungunya fever: an epidemiological review of a re-emerging infectious disease. Clin Infect Dis 49:942–948. https://doi.org/10.1086/605496
Steele KE, Reed DS, Glass PJ et al (2007) Alphavirus encephalitides. In: Dembek ZF (ed) Medical aspects of biological warfare. Office of the Surgeon General, US Army Medical Department Center and School, Borden Institute, Washington, DC, pp 241–270
Stoermer KA, Burrack A, Oko L et al (2012) Genetic ablation of arginase 1 in macrophages and neutrophils enhances clearance of an arthritogenic alphavirus. J Immunol 189:4047–4059. https://doi.org/10.4049/jimmunol.1201240
Strauss JH, Strauss EG (1994) The alphaviruses: gene expression, replication, and evolution. Microbiol Rev 58:491–562
Swanson PA, McGavern DB (2013) Portals of viral entry into the central nervous system. In: Dorovini-Zis K (ed) The blood-brain barrier in health and disease. Taylor and Francis, Abingdon, pp 23–47
Tanaka A, Tumkosit U, Nakamura S et al (2017) Genome-wide screening uncovers the significance of N-sulfation of heparan sulfate as a host cell factor for chikungunya virus infection. J Virol 91:e00432–e00417. https://doi.org/10.1128/JVI.00432-17
Taylor K, Kolokoltsova O, Patterson M et al (2012) Natural killer cell mediated pathogenesis determines outcome of central nervous system infection with Venezuelan equine encephalitis virus in C3H/HeN mice. Vaccine 30:4095–4105. https://doi.org/10.1016/j.vaccine.2012.03.076
Teng T-S, Foo S-S, Simamarta D et al (2012) Viperin restricts chikungunya virus replication and pathology. J Clin Invest 122:4447–4460. https://doi.org/10.1172/JCI63120
Teng T-S, Kam Y-W, Lee B et al (2015) A Systematic meta-analysis of immune signatures in patients with acute chikungunya virus infection. J Infect Dis 211:1925–1935. https://doi.org/10.1093/infdis/jiv049
Teo T-H, Lum F-M, Claser C et al (2013) A pathogenic role for CD4 + T cells during Chikungunya virus infection in mice. J Immunol 190:259–269. https://doi.org/10.4049/jimmunol.1202177
Thach DC, Kimura T, Griffin DE (2000) Differences between C57BL/6 and BALB/cBy mice in mortality and virus replication after intranasal infection with neuroadapted Sindbis virus. J Virol 74:6156–6161

Thach DC, Kleeberger SR, Tucker PC, Griffin DE (2001) Genetic control of neuroadapted Sindbis virus replication in female mice maps to chromosome 2 and associates with paralysis and mortality. J Virol 75:8674–8680. https://doi.org/10.1128/JVI.75.18.8674-8680.2001

Thanapati S, Ganu M, Giri P et al (2017) Impaired NK cell functionality and increased TNF-α production as biomarkers of chronic chikungunya arthritis and rheumatoid arthritis. Hum Immunol 78:370–374. https://doi.org/10.1016/j.humimm.2017.02.006

Varble A, Benitez AA, Schmid S et al (2013) An in vivo RNAi screening approach to identify host determinants of virus replication. Cell Hos Microbe 14:346–356. https://doi.org/10.1016/j.chom.2013.08.007

Venugopalan A, Ghorpade RP, Chopra A (2014) Cytokines in acute chikungunya. PLoS ONE 9:e111305. https://doi.org/10.1371/journal.pone.0111305

Villari P, Spielman A, Komar N et al (1995) The economic burden imposed by a residual case of eastern encephalitis. Am J Trop Med Hyg 52:8–13

Vogel P, Kell WM, Fritz DL et al (2005) Early events in the pathogenesis of eastern equine encephalitis virus in mice. Am J Pathol 166:159–171. https://doi.org/10.1016/S0002-9440(10)62241-9

Wang X, Li MMH, Zhao J et al (2016) Sindbis virus can exploit a host antiviral protein to evade immune surveillance. J Virol 90:10247–10258. https://doi.org/10.1128/JVI.01487-16

Wauquier N, Becquart P, Nkoghe D et al (2011) The acute phase of Chikungunya virus infection in humans is associated with strong innate immunity and T CD8 cell activation. J Infect Dis 204:115–123. https://doi.org/10.1093/infdis/jiq006

Werneke SW, Schilte C, Rohatgi A et al (2011) ISG15 is critical in the control of Chikungunya virus infection independent of UbE1L mediated conjugation. PLoS Pathog 7:e1002322. https://doi.org/10.1371/journal.ppat.1002322

Willems WR, Kaluza G, Boschek CB et al (1979) Semliki forest virus: cause of a fatal case of human encephalitis. Science 203:1127–1129

Wilson JAC, Prow NA, Schroder WA et al (2017) RNA-Seq analysis of chikungunya virus infection and identification of granzyme A as a major promoter of arthritic inflammation. PLoS Pathog 13:e1006155. https://doi.org/10.1371/journal.ppat.1006155

Wollish AC, Ferris MT, Blevins LK et al (2013) An attenuating mutation in a neurovirulent Sindbis virus strain interacts with the IPS-1 signaling pathway in vivo. Virology 435:269–280. https://doi.org/10.1016/j.virol.2012.09.008

Yasunaga A, Hanna SL, Li J et al (2014) Genome-wide RNAi screen identifies broadly-acting host factors that inhibit arbovirus infection. PLoS Pathog 10:e1003914. https://doi.org/10.1371/journal.ppat.1003914

Zhang Y, Burke CW, Ryman KD, Klimstra WB (2007) Identification and characterization of interferon-induced proteins that inhibit alphavirus replication. J Virol 81:11246–11255. https://doi.org/10.1128/JVI.01282-07

Zhang R, Kim AS, Fox JM, Nair S et al (2018) Mxra8 is a receptor for multiple arthritogenic alphaviruses. Nature 557:570–574. https://doi.org/10.1038/s41586-018-0121-3