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Protective and Pathogenic Responses to Chikungunya Virus Infection

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Abstract Chikungunya virus (CHIKV) is an arbovirus responsible for causing epidemic outbreaks of human disease characterized by painful and often debilitating arthralgia. Recently, CHIKV has moved into the Caribbean and the Americas resulting in massive outbreaks in naïve human populations. Given the importance of CHIKV as an emerging disease, a significant amount of effort has gone into interpreting the virus-host interactions that contribute to protection or virus-induced pathology following CHIKV infection, with the long-term goal of using this information to develop new therapies for safe and effective anti-CHIKV vaccines. This work has made it clear that numerous distinct host responses are involved in the response to CHIKV infection, where some aspects of the host innate and adaptive immune response protect from or limit virus-induced disease, while other pathways actually exacerbate the virus-induced disease process. This review will discuss mechanisms that have been identified as playing a role in the host response to CHIKV infection and illustrate the importance of carefully evaluating these responses to determine whether they play a protective or pathologic role during CHIKV infection.

Keywords Chikungunya virus · Alphavirus · Immunopathology · Vaccine

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Introduction

Chikungunya virus (CHIKV) is a mosquito-transmitted alphavirus belonging to the family *Togaviridae* that is responsible for epidemics of debilitating rheumatic disease associated with inflammation and destruction of musculoskeletal tissues in humans [1]. CHIKV, which can be spread by the broadly distributed mosquito vectors *Aedes aegypti* and *Aedes albopictus* [2–7], has caused sporadic epidemics of infectious arthritis in Africa and Asia. Beginning in 2004, CHIKV re-emerged in Africa and spread to throughout the Indian Ocean region, causing millions of infections in coastal Africa, islands within the Indian Ocean, India, and countries within Southeast Asia [2, 8–11]. In addition, infected travelers returning to northern Italy, New Caledonia, China, and the French Riviera initiated autochthonous outbreaks resulting from infection of local mosquito populations [12–16], illustrating the prominent role that infected travelers play in introducing CHIKV into new areas. This was further demonstrated by the introduction and subsequent epidemic of CHIKV into the Caribbean and the Americas in late 2013 [17–19]. As of October 17, 2014 the US Center for Disease Control and Prevention (CDC) reported a total of approximately 760,000 suspected and 14,000 confirmed cases of CHIKV in 36 countries or territories in the Caribbean, Central America, South America, and North America [20]. Furthermore, numerous cases of CHIKV have been brought back to the USA from the Caribbean, resulting in 11 instances of localized viral transmission in Florida as of October 21, 2014 [21].

The name chikungunya comes from the Makonde people of Tanzania where the virus was first identified in 1952–53 and loosely translates to “that which bends up” to describe the stooped posture of CHIKV-infected persons suffering from severe joint pain that characterizes infection. CHIKV-induced arthritis is most often symmetrical, accompanied by swelling, and involves multiple joints [22, 23]. Additionally,

CHIKV infection is also associated with fever, headache, chills, photophobia, muscle pain, and a petechial or macropapular rash [24, 25]. Although acute CHIKV infection is generally self-limiting after 7–14 days, continuing joint pain and lethargy are observed in about a third of patients for months, and in over 10 % of patients, these sequela may persist for years [12, 23, 26–30]. Analysis of the 2004–2007 epidemic suggest that the re-emergence of CHIKV is also cause for concern due to increased morbidity and mortality associated with infection [31, 32]. Greater numbers of CHIKV-infected persons developed the more severe forms of the disease including neurological complications and fulminant hepatitis, while maternal-fetal transmission associated with neonatal encephalopathy was also reported [31, 33–36].

The host immune system plays a complex role in the pathogenesis of CHIKV-induced disease. There is abundant evidence that components of the innate immune system, including the type I interferon system, play an essential role in protecting from CHIKV-induced disease, while CHIKV-specific neutralizing antibodies mediate long-term immunity to CHIKV. However, it is also clear that components of the host immune response can also play an immunopathologic role in the pathogenesis of CHIKV-induced arthritis [37•, 38–40, 41•]. Therefore, the focus of this review is to discuss the field's current understanding of the host innate and adaptive immune response to CHIKV, with an emphasis on differentiating between those aspects of the response that mediate protection or contribute to virus-induced immune pathology.

Host Cellular Processes in Response to Chikungunya Infection

CHIKV infection at the cellular level is a cytopathic event with rapid onset of apoptosis in vivo and in vitro [42–46]. Apoptosis is thought to be the result of innate immune processes as well as the general block in host-cell translation induced by CHIKV and other alphaviruses [47–50]. Although apoptosis was suggested to be a host-protective mechanism to limit virus production and spread, it is now recognized that the host apoptotic machinery can be hijacked by many different viruses including alphaviruses to the detriment of the host [51, 52]. The release of apoptotic blebs from dying cells has been shown to increase the spread of CHIKV from apoptotic infected cells to uninfected neighboring cells as well as macrophages in vitro [53]. This process would allow for cell-to-cell spread of the virus without exposure to extracellular immune cells and mediators, such as antibody. Additionally, in this system, macrophages were able to be infected following phagocytosis of CHIKV-containing blebs. Thus, apoptosis may serve as a mechanism for allowing CHIKV infection of cells that are non-permissive to direct viral entry as well as allowing the virus to evade host immune mechanisms. Finally, a recent high-throughput screen of a library of various kinase inhibitors in a human cell line identified several compounds which rather than limiting viral replication, instead decreased viral cytopathic effect, likely by targeting components of the apoptotic pathway [54]. Further work will need to be done to characterize the role for these apoptotic mechanisms and inhibitors in vivo.

Another cellular process which has been investigated in the context of alphaviruses and was shown to play both antiviral and proviral roles is the process of autophagy [55, 56]. In particular, CHIKV infection has been shown to induce autophagy in human cells where the process dramatically increases CHIKV replication [57]. Yet in the mouse model, autophagy appears to limit CHIKV pathogenesis. Mouse embryo fibroblast (MEF) cell cultures deficient for autophagy protein (ATG) 5, a E3 ubiquitin ligase necessary for autophagy, exhibited a significant increase in CHIKV-induced cell death compared with wild-type MEFs [58]. Taken together, these studies indicate that there may be some species specificity to the autophagy mechanism and response following CHIKV infection. Indeed, experimental evidence exists indicating that the human autophagy receptor NDP52 interacts with the CHIKV non-structural protein 2 (nsP2) to promote viral replication while no such interaction occurs with the mouse orthologue of NDP52 [59•]. In addition to species specificity, these researchers showed that different autophagy receptors in human cells have distinct roles based on interactions with different viral components. In contrast to the proviral outcome of the NDP52-nsP2 interaction, the p62 autophagy receptor appears to interact with ubiquitinated CHIKV capsid protein to target it for degradation thus providing a cytoprotective mechanism for the host [59•].

Interestingly, induction of the autophagy pathway by CHIKV infection delays or mitigates the apoptotic anti-CHIKV response [58]. These researchers found that CHIKV activates both the endoplasmic reticulum (ER) stress and oxidative stress pathways, albeit through distinct mechanisms, to induce autophagy. In Atg16L^{HM} mice, which have decreased levels of autophagy, CHIKV infection resulted in enhanced apoptosis and subsequent increased lethality. These experiments were critical in examining the mechanisms of autophagy in CHIKV infection and elucidating a model wherein autophagy plays a protective role in CHIKV pathogenesis via its modulation of the apoptotic pathway.

Heat shock proteins (HSPs) are involved in the cellular response to stress such as protein folding/unfolding, protein transport, and protection against apoptosis. In addition, extracellular HSPs can stimulate professional antigen-presenting immune cells. HSP90 was recently shown to promote CHIKV infection as numerous drugs that inhibit HSP90 also inhibit viral replication and limit disease both in vitro and in a mouse model [60]. This same set of experiments identified a direct interaction between CHIKV nsP3/nsP4 and HSP90. Furthermore, HSP90 was shown to stabilize CHIKV nsP2 and promote viral replication in vitro [61]. Another protein, HSP70,

was recently shown to bind CHIKV virus in both HEK-293 and Vero-E6 cells [62]. These studies represent the earliest examination of the role of HSPs in CHIKV infection, and it can be assumed that much more evidence of interactions between the virus and these stress response molecules will be forthcoming.

Early Innate Responses to CHIKV

The mammalian innate immune system plays a crucial role in protecting the host from viral infections, by initially sensing the virus, limiting viral replication, and ultimately shaping the nature of the adaptive immune response. Therefore, much of the work examining protective and pathologic immune responses to CHIKV have been focused on understanding how the innate immune response protects from CHIKV infection [63–65], with a significant amount of work focused on the type I IFN system and its role in protecting from CHIKV-induced disease. However, in addition to playing a protective role, there is also evidence that components of the innate immune system contribute to CHIKV-induced immune pathology. Therefore, any discussion of innate immunity's role in the pathogenesis of CHIKV-induced disease should take into account both the protective and the pathologic aspects of the disease process.

Type I Interferon-Mediated Protection From CHIKV-Induced Disease

In the vertebrate host, the type I interferon (IFN) pathway is critical for controlling viral replication and pathogenesis during the early stages of CHIKV infection. IFNs are capable of playing a direct antiviral role by limiting viral replication/dissemination or they may regulate pro-immune cytokine expression leading to a potent host immune response. Mice deficient for both the IFNα and IFNβ receptor ($IFNAR^{-/-}$) develop severe CHIKV-induced disease associated with increased viral titers and central nervous system (CNS) involvement [66, 67]. Given the clear importance of the IFN pathway in protecting from CHIKV-induced disease, a significant amount of work has centered on identifying points of interaction between CHIKV and the host type I IFN pathway.

In vitro work with both human peripheral blood mononuclear cells (PBMCs) and mouse dendritic cells suggests that CHIKV does not directly interact with pattern recognition receptors on hematopoietic cells to initiate the type I IFN cascade. Rather, it is cooperation between multiple host adaptor molecules on CHIKV-infected fibroblasts that appear to initiate production of type I IFN following CHIKV infection [67]. Induction of this type I IFN response occurred via the CARD adaptor inducing IFNβ (MAVS, also referred to as Cardif or IPS-1) sensor acting downstream of the RNA sensors MDA5 and RIG-I to limit CHIKV infection [67, 68]. Mouse embryo fibroblast (MEF) cultures prepared from $MAVS^{-/-}$ animals were more sensitive to CHIKV infection than wild-type MEFS, and this correlated with a lack of IFNβ production in $MAVS^{-/-}$ MEFS. However, $MAVS^{-/-}$ mice were not as sensitive to CHIKV infection as $IFNAR^{-/-}$ mice, suggesting that while MAVS plays a critical role in inducing a protective type I IFN response following CHIKV infection, other mechanisms are involved in inducing the type I IFN response. Indeed, the same set of experiments showed that the presence of myeloid differentiation primary response protein 88 (Myd88), the adaptor for multiple toll-like receptors (TLRs) and interleukin-1β, may serve to limit CHIKV viral replication in vivo, presumably via type I IFN induction. The authors hypothesized that endosomal Myd88-dependent TLRs may be engaged as a result of immune cell phagocytosis of infected cells which would provide a source of viral proteins for recognition in hematopoietic cells. The fibroblast as the major type I IFN producer in CHIKV infection sets CHIKV apart from other closely related alphaviruses such as RRV, Sindbis virus, and VEE which are able to both infect and induce type I IFN production in mouse dendritic cells [69, 70].

Due to the critical role IFNs have in protecting against CHIKV-induced disease, many studies have focused on identifying the downstream interferon stimulated genes (ISGs) that mediate antiviral activity against CHIKV. A large-scale study which looked at gene products induced by the type I IFN in response to a wide range of viruses included CHIKV [71•]. This study identified the double-stranded RNA-specific adenosine deaminase (ADAR) as an enhancing effector for CHIKV replication. In contrast, multiple genes with significant inhibitory effects on CHIKV replication were also identified: SLC15A3 (Mitoferin-2), SLC25A28, HPSE (Heparanase), C6orf150 (Cyclic GMP-AMP synthase), UNC93B1 (regulates nucleotide-sensing TLRs), DDX58, (retinoic acid-inducible gene 1; RIG-I), P2RY6 (Pyrimidinergic Receptor P2Y, G-Protein Coupled, 6), and interferon regulatory factor 1 (IRF-1). Of these with inhibitory effects, P2RY6, SLC15A3, and SLC25A28 were specific to CHIKV across all of the viruses tested. These findings led to screening of multiple ISGs and their contribution to CHIKV infection and disease.

Initially, expression of the ISG viperin was shown to be upregulated in human fibroblast cultures following CHIKV infection [68]. Mice deficient for viperin had higher viremia and more severe joint inflammation compared with wild-type mice following CHIKV infection [72•]. ISG15, a ubiquitin-like molecule, is protective during CHIKV infection of neonatal mice, although it was found to act in a non-classical, conjugation-independent manner [73•]. The ISG known as bone marrow stromal antigen 2 (BST-2) or tetherin was thought to act as a host restriction factor by tethering budding CHIKV virus-like particles to the host-cell membrane thus limiting viral spread [74•]. In vivo, BST-2 deficiency results

in increased CHIKV titer at the site of inoculation leading to higher levels of viremia and increased tropism for lymphoid tissues in the face of suppressed innate inflammatory responses [75•]. However, BST-2's effects can also be antagonized by the nsP1 protein of CHIKV which is able to downregulate BST-2 expression [74•]. These findings highlight the fact that the type I IFN system is a critical protective component of the innate immune response against CHIKV infection of vertebrates, although the ability of the virus to avoid or suppress these responses prevents them from completely controlling CHIKV replication or protecting from disease [66, 67, 73•, 76•, 77•, 78].

The Inflammatory Response to CHIKV and Mediators of Virus-Induced Immune Pathology

Although studies of the type I IFN system have provided important insights into the protective aspects of the innate immune response, there is also abundant evidence that components of the host innate and/or inflammatory response play a pathologic role during CHIKV infection. Numerous other host cells and molecules respond to protect the host and limit CHIKV replication and damage, although these responses are often poorly regulated and/or inappropriate and lead to the immune mediated pathology associated with CHIKV infection.

Macrophages appear to be the main infiltrating cell type in infected tissues following alphaviral infection [78–80, 81•, 82] and have been implicated in both protective and pathogenic mechanisms in CHIKV infection. Mice treated with clodronate liposomes to deplete macrophages showed reduced foot swelling and prolonged viremia suggesting that macrophages contribute to CHIKV-induced damage and disease, while contributing to viral control [78]. Furthermore, mice treated with bindarit, an inhibitor of monocyte chemotactic proteins (MCPs), were protected from joint and muscle tissue inflammation following CHIKV infection in a mouse model [83]. MCPs regulate macrophage migration to sites of inflammation. MCP-1 (CC chemokine 2 CCL2) is significantly elevated in early, acute CHIKV-infected human and non-human primate serum, as well as infected CHIKV mouse tissues [82, 84]. Interestingly, in mice lacking the receptor for CCL2, arthritic disease was substantially enhanced both quantitatively and temporally compared to wild-type mice without an increase in viral load or persistence [85]. In this model, the monocyte/macrophage infiltrate was replaced by a severe neutrophil and subsequent eosinophil infiltration. Thus, $CCR2^{+}$ monocytes/macrophages appear to be critical for dampening inflammation and preventing excessive musculoskeletal pathology and following CHIKV infection, potentially by limiting a more severe neutrophil-dominated response. Finally, macrophages present in CHIKV-induced musculoskeletal inflammatory lesions express genes consistent with an M2 macrophage-like activation pattern [41•]. M2 macrophages, also known as alternatively activated macrophages, are thought to have anti-inflammatory, immunoregulatory functions and exhibit a wound-healing phenotype [86, 87]. The same work demonstrated that genetic deletion of arginase 1 (Arg1), an immunoregulatory enzyme associated with M2 macrophages or macrophage-like cells, increased Ross River virus (RRV) clearance and limited tissue pathology in vivo [41•]. Therefore, M2 macrophages may actually promote the development of CHIKV persistence in some individuals, which may contribute to the development of chronic CHIKV disease. Furthermore, macrophages appear to be able to harbor CHIKV infection in macaques, which suggests that these cells may be responsible for the chronic symptoms observed in a percentage of human CHIKV cases [71•].

Natural killer (NK) cells are present in the inflammatory environment in many viral infections including CHIKV [78, 81•, 88•], although their role in the disease process is unclear. In general, NK cells are one of the earliest effector cells to respond to viral infection where they exert their effects through IFNγ production, cytokine secretion, and cytotoxic ability [83, 89]. In fact, NK cells were shown to be highly activated in the serum during human CHIKV infection, and they were not affected by the lymphopenia impacting other lymphocyte populations early after CHIKV infection [88•]. Ng et al. demonstrated high levels of IL15, a stimulating cytokine for NK cells and T cells, in the serum of acutely infected CHIKV patients [90]. Finally, one group recently characterized the NK cell response to CHIKV infection using CHIKV-infected human cells [91]. CHIKV infection results in an early, transient shift of NK-cell phenotype and function that correlated with viral load. The study identified clonal expansion of NK cells that express both CD94/NKG2C and inhibitory receptors for HLA-C1 alleles, where these highly mature NK cells exhibited high preference for cytotoxicity coupled with diminished IFN-γ production. However, functional impact of these cells on the CHIKV disease process is still unknown, and there is clearly a need for further explorations into the role NK cells play in CHIKV infection.

In contrast to other alphaviruses, relatively little work has been done on the role of dendritic cells (DCs) in CHIKV infection. Unlike RRV [69], Sindbis virus [92], and VEE [93], there is no evidence that CHIKV can infect DCs in humans, non-human primates or mice [42, 50, 82]. However, a study of CHIKV-infected patients who had been classified as "recovered" or "chronic" at a year postinfection identified dendritic cells as robust responders to early CHIKV infection in humans [88•]. Work in our lab demonstrated that the dendritic cell immunoreceptor (DCIR) plays a host-protective role in CHIKV infection of mice [64]. DCIR-deficient mouse bone marrow-derived DCs produced increased cytokines IL6 and IL10, and $DCIR^{-/-}$ mice had exacerbated disease and musculoskeletal pathology following CHIKV infection.

Additionally, there is some controversy over the ability of human blood monocytes to become infected with CHIKV. One study looked at blood samples taken from human CHIKV patients and showed that blood monocytes appear to be major targets of CHIKV infection during the viremic phase of disease [94]. The authors further supported their findings using an in vitro infection model of CHIKV infection of healthy human blood samples. In contrast, another group was not able to demonstrate infection of human peripheral blood mononuclear cells (PBMCs), as well as purified monocytes, DCs, and $CD4^+$ T cells [42]. The discrepancies between these two experiments suggests that the involvement of these cell types in CHIKV infection may be more complicated than that observed for other alphaviruses.

Adaptive Immune Response

The role of the adaptive immune response in CHIKV clearance and pathogenesis has not been extensively studied. However, mice lacking T and B cells ($RAG2^{-/-}$) have persistent, high-level viremia with no evidence of inflammation within infected tissues [95•], suggesting that the adaptive immune response is critical for viral control and elimination. Additionally, $Rag1^{-/-}$ mice have long-term persistent levels of CHIKV RNA in infected tissues, joints, and serum further implicating the adaptive immune response in control of CHIKV infection [39].

T Lymphocytes

In human acute CHIKV infection, acute lymphopenia has been observed [96]. $CD8^+$ T cells predominate in the early stages of the disease with $CD4^+$ T cells mediating the adaptive response at later times postinfection [65]. Both $CD4^+$ and $CD8^+$ T cells have been shown to infiltrate CHIKV-infected tissues in mouse models of infection [78, 81•]. While the role of $CD8^+$ T cells in CHIKV pathogenesis remains unclear, $CD4^+$ T cells were recently shown to mediate pathogenesis during CHIKV infection in mice independent of changes in viral titer and IFNγ production [95•]. $CD4^{-/-}$ mice had lower levels of anti-CHIKV antibody with reduced neutralizing activity, although this did not affect their ability to control CHIKV infection [97]. While these studies provided evidence that T cells contribute to CHIKV protection and pathogenesis, characterization of the type of T cells responsible and the mechanism by which these cells contribute to CHIKV infection requires further study.

B Lymphocytes and Antibody

The antibody response to CHIKV has been shown to be important in human and mouse models. While T cells play a role in modulating the inflammatory response, they do not appear to play a pivotal role in limiting viral replication [95•]. On the other hand, mice that lack B cells develop viremia that persists for over a year and exhibit increased CHIKV acute disease suggestive of a role for B cells in viral clearance and control [97]. Furthermore, passive transfer of human anti-CHIKV antibody is sufficient to diminish or stop CHIKV infection in adult $IFNAR^{-/-}$ and neonatal wild-type mice when used prophylactically [98]. In another study, combinations of mouse anti-CHIKV monoclonal antibodies were sufficient to protect $IFNAR^{-/-}$ mice against lethality when given therapeutically 24-h postinfection [99]. Taken together, these findings suggest that anti-CHIKV antibodies are the major correlates of immunity and the induction of a strong B cell response is a critical component of CHIKV vaccine candidates.

Chikungunya Vaccine Design

Due to the explosive nature of CHIKV epidemics, vaccine design is critical from both a public health and economic standpoint. Although generally not fatal, the painful debilitating nature of CHIKV infection and its impact on productivity were sharply illustrated in the 2004–2007 outbreak. Up to 72 % of patients in India suffered from arthralgia that persisted for up to 1 month following CHIKV infection [100] and within a single epidemic region, approximately 65 % of disability within the population resulted from CHIKV infection [101]. However, evidence for immune-mediated pathology associated with CHIKV makes proper vaccine design imperative to avoid elements which may exacerbate disease and pathology in CHIKV-infected persons.

There have been several promising attempts to develop a vaccine against CHIKV, and while a comprehensive review of the different strategies is beyond the scope of this review, we will highlight a few examples of the different approaches that have been taken. Live attenuated CHIKV vaccines hold promise due to their relatively low production costs and their ability to elicit protective immunity with a single immunization. An attenuated CHIKV strain, designated 181/25, was produced through serial passages through MRC-5 cells and tested in both mice and non-human primates and shown to offer protection against wild-type virus challenge [102]. However, in phase II trials, a small percentage of vaccinated individuals developed a mild, transient arthralgia following vaccination [103]. In addition to those adverse effects, the virus was passaged in uncertified cell cultures during production, and attenuation of the virus was found to be the result of only two point mutations [104]. In an attempt to increase the safety of attenuated vaccine viruses, another group deleted a large portion of the nsP3 gene or the entire 6K gene [105]. Vaccine preparations consisted of the mutated viruses produced as viral particles or DNA-launched infectious genomes. Mice that received a single vaccination of either mutant vaccine had high levels of neutralizing antibody, a strong T cell response, and were

protected against high-dose virus challenge. A second dose of the vaccine increased immunogenicity. In addition to these classical attenuation approached, another group inserted the encephalomyocarditis (EMCV) virus internal ribosome entry site (IRES) into the CHIKV subgenomic promoter, which both attenuated the virus in mammals and negated translation of the structural proteins in arthropod cells to prevent replication in mosquitoes [106]. In different mouse models, the vaccine was highly attenuated, and a single dose proved immunogenic and efficacious. Additionally, this vaccine was protective in a non-human primate challenge model [107].

One alternative to live attenuated CHIKV vaccines is the use of virus-like particles (VLPs) for vaccination. These structures mimic the organization and conformation of the authentic native virus, but lack the non-structural replication machinery and are therefore noninfectious. Noranate et al. characterized CHIKV VLPs which had been codon optimized and derived from a human cell line [108]. These VLPs were able to bind antibodies of mouse and convalescent human serum in an ELISA assay suggesting that the antigenicity of VLPs may be similar to that of wild-type CHIKV. Furthermore, CHIKV VLP vaccination of non-human primates resulted in high-titer neutralizing antibody production and protection against viremia following CHIKV challenge in both the non-human primates and mice receiving antibody transfer [109]. Additionally, the VLP vaccine was recently shown to be well tolerated by human vaccinates and elicited neutralizing antibodies which persisted for at least 6 months following a prime/boost regiment [110].

In addition to live attenuated and VLP vaccines, several groups have produced CHIKV vaccine candidates using a poxvirus vector modified vaccinia virus Ankara (MVA) expressing the CHIKV structural proteins [111]. The vaccine was highly immunogenic and protective in a mouse high-dose challenge model. Other groups have used the MVA vector to express only parts of the CHIKV structural protein region and afforded protection to different mouse models against lethal challenge [112, 113].

Conclusion

With the introduction of chikungunya virus into the Americas, combined with the ongoing spread of the virus in Southeast Asia and the South Pacific, interest has been renewed in understanding more about this virus and the pathogenesis of CHIKV disease. Understanding the protective and pathogenic mechanisms that are initiated when virus meets host is where the game is won or lost, and information on these processes will inform the development of safe and effective CHIKV vaccines and therapeutics. It is clear from the selection of work reviewed herein that CHIKV disease is a complicated process involving numerous interactions between the host and virus. Furthermore, work from a number of groups indicates that some of the same processes that can protect from CHIKV-induced disease may also contribute to virus-induced pathology. Therefore, further work is clearly needed to determine how perturbations in these processes impact CHIKV-induced disease, with the long-term goal of using this information to design safer, more effective, CHIKV vaccines, or anti-inflammatory therapies.

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Compliance with Ethics Guidelines

Conflict of Interest Kristin M. Long and Mark T. Heise declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent For all referenced works involving animals where either Dr. Long or Dr. Heise were listed as authors, all studies were performed in accordance with the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee guidelines and with IACUC approval.

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