Animal Models of Chikungunya Virus Infection and Disease

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Chikungunya virus (CHIKV) is a reemerging alphavirus that causes acute febrile illness and severe joint pain in humans. Although acute symptoms often resolve within a few days, chronic joint and muscle pain can be long lasting. In the last decade, CHIKV has caused widespread outbreaks of unprecedented scale in the Americas, Asia, and the Indian Ocean island regions. Despite these outbreaks and the continued expansion of CHIKV into new areas, mechanisms of chikungunya pathogenesis and disease are not well understood. Experimental animal models are indispensable to the field of CHIKV research. The most commonly used experimental animal models of CHIKV infection are mice and nonhuman primates; each model has its advantages for studying different aspects of CHIKV disease. This review will provide an overview of animal models used to study CHIKV infection and disease and major advances in our understanding of chikungunya obtained from studies performed in these models.

Keywords. Chikungunya; *Alphavirus*; animal models; nonhuman primates; mouse models.

Chikungunya virus (CHIKV) is a mosquito-transmitted positive-sense RNA virus in the *Alphavirus* genus in the family *Togaviridae*. The hallmark symptoms of CHIKV infection in humans are debilitating arthralgia that affects the peripheral joints, causing intense pain and swelling, high fever, and sometimes rash [1]. These symptoms may resolve within a few days, but many patients experience recurring disabling arthritic pain for months to years [2, 3]. The long-term chronic arthralgia can have an enormous impact on the individual's quality of life [4, 5]. CHIKV can cause widespread outbreaks in areas where there are competent mosquito vectors and CHIKV-naive populations, as demonstrated by the recent CHIKV outbreak in the Americas. The first reported cases of local CHIKV transmission in the Americas occurred in Saint Martin, a Caribbean island [6]. Since then, CHIKV has rapidly spread throughout the Caribbean and to countries in Central and South America causing >1.7 million cases [7]. Following a large outbreak, CHIKV may disappear for years to decades, most likely because of a lack of susceptible hosts [8]. At a later time, CHIKV can reemerge in regions that no longer have preexisting CHIKV immunity [8]. CHIKV infects other species, such as nonhuman primates, that can maintain the virus in a sylvatic enzootic transmission cycle involving forest-dwelling *Aedes* mosquitoes.

HUMAN CHIKUNGUNYA

Acute chikungunya symptoms in humans appear 3–12 days after a bite by an infected mosquito [9]. The most common acute symptoms are sudden onset of fever, polyarthralgia, headache, myalgia, rash, and fatigue [1, 9]. The arthralgia usually occurs in >1 joint, particularly in peripheral joints (wrists, fingers, ankles, and toes) [10]. Viral titer peaks within the first few days of symptom onset and can persist as long as 12 days [9]. Newborns and seniors (>60 years), who tend to have higher viral load, are at risk for severe acute infection and usually exhibit more-severe, atypical symptoms of CHIKV infection, such as skin lesions, encephalitis, seizures, and blindness [1, 10–12]. The case-fatality rate following CHIKV infection is relatively low (approximately 1:1000), and many of these cases involved elderly patients [13]. The convalescent phase of CHIKV infection is associated with resolution of fever and viremia and induction of adaptive immunity. Although other acute symptoms resolve, joint and muscle pain may remain for weeks to years [2, 14, 15]. Roughly 43%–75% of CHIKV-infected patients experience persistent/recurring symptoms, including fatigue and joint pain, stiffness, and swelling, for about 2 years following CHIKV infection [3]. Risk factors for developing chronic joint pain after acute CHIKV infection include increased age and disease severity during the acute stage [14–16]. The ability of CHIKV to replicate and persist in the joints and muscle tissues over the long term is still debated in the field. CHIKV antigen was detected in synovial and muscle tissue of patients experiencing chronic arthritic pain [14, 17].

Although this is suggestive of persistent CHIKV replication, more research is needed to increase the understanding of chronic chikungunya-induced joint and muscle pain. Despite its increasing spread worldwide, many details of chikungunya pathogenesis are not well understood. Therefore, there is increasing

interest and need for use of animal models to study CHIKV infection.

MOUSE MODELS

Overview
Mouse models are a powerful resource for studying chikungunya pathogenesis and as early-stage testing platforms for evaluating CHIKV vaccines and therapeutics. Several attributes make mice attractive model systems for studying CHIKV, including (1) their relatively low cost; (2) the ease of housing, owing to their small size; (3) the availability of large panels of mouse-specific reagents, such as immune reagents, for quantifying the host response to CHIKV infection; (4) the availability of inbred lines, which allows genetically identical sets of mice to be used to assess phenotypic reproducibility or to test the response against different treatments in the same line; and (5) the availability of genetically modified mice, such as single-gene-knockout animals, which allow the role of specific host genes or pathways during CHIKV infection to be evaluated. These features have led to the wide use of various mouse models for evaluating viral virulence determinants, characterizing the host pathways that play protective or pathologic roles during CHIKV infection, and assessing the efficacy and safety of early stage CHIKV vaccines and therapeutics. However, while mouse models are powerful resources, existing mouse models also have limitations when considering their ability to model every aspect of CHIKV infection in humans. There are instances in which mice do not reproduce specific aspects of human CHIKV infection, owing to a lack of functional conservation between mouse and human genes, and existing mouse models do not fully reproduce key aspects of CHIKV disease, including maternal/neonatal transmission, enhanced CHIKV disease in elderly individuals, or chronic chikungunya, and opportunities exist to develop new model systems that better reproduce these aspects of human CHIKV disease.

Acute Infection Models
Acute CHIKV disease mouse models can roughly be broken down into 3 categories: lethal neonatal challenge models, immunocompromised models of lethal disease, and CHIKV arthritis/myositis models. All 3 models have made valuable contributions to our understanding of chikungunya pathogenesis and have concurrently served as early stage testing platforms for evaluating candidate CHIKV vaccines and therapies.

Lethal Neonatal Challenge Models
Neonatal mice are highly susceptible to CHIKV infection and, prior to modern cell culture techniques, were standard tools for the isolation and amplification of viruses such as CHIKV [18]. Neonatal mice, which develop lethal encephalitis, have been used to model human neonatal infection and provide a system for studying viral and host factors that contribute to severe disease in neonates [19, 20]. In addition to their usefulness as pathogenesis models, neonatal mice are sensitive tools for testing the efficacy of CHIKV-specific polyclonal and monoclonal antibodies (mAbs) or other therapeutics because of their high sensitivity to CHIKV infection. Furthermore, because of their immature immune systems, neonatal mice cannot be used to directly test vaccine efficacy, but they are useful for testing the safety of live attenuated vaccines [21]. However, the immature state of their immune systems and the fact that they rapidly succumb to lethal CHIKV disease significantly limit their usefulness for studying the pathogenesis of CHIKV-induced arthritis or for testing CHIKV vaccines.

Immunocompromised Models of Lethal Disease
The type I interferon (IFN) system is essential in protecting from systemic CHIKV; mice lacking a functional type I IFN receptor or other key components of the type I IFN pathway (eg, IRF3/IRF7) are highly susceptible to CHIKV infection [19, 22–24]. Therefore, these mice are useful tools for studying the role of the type I IFN system in CHIKV pathogenesis and are highly sensitive lethal challenge models for testing the efficacy of anti-CHIKV Abs or the safety and efficacy of CHIKV vaccines, as illustrated by the studies of Plante et al and Pal et al [25, 26]. However, type I IFN receptor–deficient mice rapidly die of CHIKV infection, which limits their usefulness for studying chikungunya pathogenesis.

Arthritis/Myositis Models
Immunocompetent adult mouse models of CHIKV-induced arthritis are valuable systems both for studying the pathogenesis of CHIKV-induced arthritis and for testing CHIKV vaccines and therapies. Subcutaneous CHIKV infection in the footpad of C57BL/6 mice results in a biphasic swelling response in the inoculated foot, with peaks occurring approximately 3 and 7 days after infection. The inoculated foot also develops severe arthritis, tendonitis, and fasciitis, while the contralateral foot does not exhibit swelling, and inflammatory changes are relatively milder [27, 28]. By using gene-specific knockout mice, a number of groups have begun to identify host factors that play either protective or pathologic roles during CHIKV arthritis, including IFN-stimulated genes such as ISG15 and IFIT1 [20, 29]. Likewise, knockout mouse studies have proven useful for identifying host genes and cell types, such as CD4^{+} T cells, that drive acute CHIKV-induced pathology [30, 31]. In addition to enhancing our overall understanding of chikungunya pathogenesis, these models have been useful for testing vaccines and therapies for their ability to protect against CHIKV-induced arthritis [32, 33].

Chronic/Persistent Models
The progression to chronic musculoskeletal pain and inflammation occurs in up to two thirds of CHIKV-infected patients. The mechanisms of chronic chikungunya pathogenesis are not well understood. In a limited number of human studies, CHIKV antigen and RNA have been detected in synovial tissue biopsy specimens collected from patients with chronic joint pain, with CHIKV antigen detected in perivascular macrophages [14]. CHIKV antigen also was detected in muscle satellite cells in muscle biopsy tissue

specimens collected from a patient during a relapse of chronic musculoskeletal pain [17].

Accordingly, mouse models have begun to be used to investigate the persistence of CHIKV infection and its association with chronic disease. C57BL/6 mice inoculated in the footpad with a clinical isolate of CHIKV showed detectable viral RNA in a variety of tissues at early time points after infection [30]. By 4 weeks after infection, CHIKV RNA was cleared from many tissues but remained detectable in joint-associated tissues and lymphoid tissue, suggesting that these tissues support chronic CHIKV infection. In this study, viral RNA was detected in joint-associated tissues for at least 16 weeks after infection. The persistence of CHIKV in these mice was associated with persistent synovitis, suggesting that chronic CHIKV infection may cause chronic joint inflammation [30]. Although isolation of infectious virus from these tissues during the chronic phase has so far been unsuccessful, evidence suggests that CHIKV persists in these tissues by low-level continuous replication [31]. It is currently unclear whether CHIKV acquires adaptive mutations during infection of mice that contribute to the development of persistence [31, 34]; an in-depth analysis of CHIKV sequence evolution in different tissue compartments during the acute and chronic phases of infection of wild-type mice is warranted.

Mouse models are also being used to define immunological mechanisms that contribute to the control of CHIKV infection and how these may be altered during persistence. Work by several groups has defined important roles for the adaptive immune response, particularly the B-cell response, in control of CHIKV infection [30, 31, 34–36]. While the adaptive immune response appears to limit but not fully clear CHIKV from musculoskeletal tissues, B cell– and T cell–deficient mice do not die of CHIKV infection despite supporting persistent viremia and elevated viral burdens in a variety of tissues [30, 31, 34–36]. These data suggest that innate antiviral responses limit CHIKV replication independent of adaptive immunity.

As highlighted above, mouse models can be exploited to investigate both virologic and immunologic mechanisms that influence chronic CHIKV infection and disease. However, these studies have been performed in a narrow range of mouse strains, and there remains a critical need to improve mouse models to reflect the diverse manifestations of chronic CHIKV disease, which range from post-chikungunya musculoskeletal disorders to post-chikungunya de novo chronic inflammatory rheumatism [37]. In support of this goal, preliminary studies in the Collaborative Cross (CC), a highly diverse mouse genetic reference population [38], found that different CC mouse lines exhibit a diverse array of responses to CHIKV infection, including CC mouse lines that are resistant or highly susceptible to virus-induced swelling and lines that exhibit disseminated inflammation following CHIKV infection (K. Plante, J. Plante, M. T. Ferris, and M. T. Heise, unpublished data). Furthermore, the diversity of CHIKV disease outcomes observed in the CC has led to the initial identification of quantitative trait loci associated with variation in CHIKV disease susceptibility within the CC, setting the stage for future studies analyzing whether genes identified in the CC have relevance to human disease. In addition to a need for models that better represent the diverse CHIKV disease outcomes observed in humans, a need also exists for improved models for studying the impact of aging on CHIKV disease, since aged individuals may be at greater risk for developing severe acute or chronic CHIKV disease [14–16].

NONHUMAN PRIMATE (NHP) MODELS OF CHIKV INFECTION

The first experimental NHP CHIKV infection models were tested in the 1960s, using rhesus macaques (*Macaca mulatta*) and bonnet macaques (*Macaca radiata*) [39, 40]. In these early studies, CHIKV infection was assessed by the development and duration of viremia, along with the presentation of clinical symptoms, including fever. Recently, NHP CHIKV infection models have been used to study chikungunya pathogenesis and as preclinical models to evaluate the efficacy of vaccines and immunotherapeutics. The breadth of models available has expanded to include new NHP models using cynomolgus macaques (*Macaca fascicularis*) and further development of the rhesus macaque model to recapitulate CHIKV infection in aged and pregnant populations [41–43].

Cynomolgus Macaque Model of CHIKV Infection

CHIKV infection of cynomolgus macaques results in acute disease signs and viremia that are similar to those observed in humans, followed by viral persistence in some tissues up to 3 months after infection [41]. High-dose infection (10^8 plaque-forming units) resulted in meningoencephalitis and may recapitulate neurologic disease in humans [41]. Viremia developed within 1 day after infection, lasting until 6–7 days after infection, with tissue dissemination observed 2 days after infection and peak viral RNA loads reached 6 days after infection. Notably, virus remained detectable in lymphoid organs until 3 months after infection and in synovial and muscular tissues until 1.5 months after infection, and infectious virus was recovered from the spleen, liver, and muscle as late as 44 days after infection, indicating that the virus persists for long periods [41]. The level and duration of viremia and associated disease was dependent on the infecting dose of CHIKV. During human CHIKV infection, leukopenia is also reported to occur; this phenomenon was recapitulated in the cynomolgus macaque model, with significant monocytopenia, lymphopenia, granulocytosis, and thrombocytopenia occurring in infected animals as compared to uninfected control animals [41]. Limited aspects of the immune response to CHIKV-LR in cynomolgus macaques have been studied. The plasma levels of a number of cytokines and chemokines, including IFN-α/β, interleukin 6, monocyte chemoattractant protein 1, and IFN-γ, correlated with peak levels of viremia [41]. Both immunoglobulin M and immunoglobulin G CHIKV-specific Ab responses were detectable in this model of CHIKV, and these Abs are capable of recognizing similar epitopes as observed in humans [44, 45]. This model not only recapitulates

common characteristics of CHIKV infection, but also provides evidence for CHIKV persistence within NHP tissues.

Rhesus Macaque Model of CHIKV Infection

CHIKV infection of rhesus macaques resulted in peak viremia levels 1–2 days after infection, lasting to an average of 6 days after infection [42, 43, 46, 47], and high fever that coincided with rash during the first week of infection [43]. Studies in rhesus macaques completed by Pal et al by using CHIKV-specific mAbs generated interesting insights into dissemination routes of CHIKV [47]. In these studies, CHIKV-infected rhesus macaques were treated with anti-CHIKV or control mAbs 1 and 3 days after infection. Differences in viral loads between the anti-CHIKV mAb–treated group and control group at 7 days after infection led to the speculation that CHIKV spreads from the site of infection, in the arm, into the blood, where it then goes on to infect the spleen and heart, followed by establishment in distal joints and muscles, the lungs, and kidneys [48]. This study exhibits how the rhesus macaque model of CHIKV infection can be used to answer mechanistic questions about chikungunya pathogenesis and serve as a preclinical model for testing novel anti-CHIKV therapeutics.

The immune responses to CHIKV have been characterized in the rhesus macaque model, providing important insight into correlates of protection during human infection. Frequency of innate immune cells in the peripheral blood of infected animals revealed an increase in monocytes/macrophages and all dendritic cell subsets after infection [43]. In general, T-cell and B-cell proliferative responses peaked 10–14 days after infection, with a second burst of proliferation occurring 28 days after infection in the memory B-cell subset [43]. The B-cell response was followed by a CHIKV-specific Ab response peaking around 21 days after infection [43]. We are still lacking key pieces of information about the host-specific immune response to CHIKV, especially adaptive immune components, but these studies provide insight into important CHIKV-specific immune control mechanisms.

Aged NHP Model of CHIKV Infection

During the CHIKV outbreak in La Reunion, individuals within the aged population (ie, those >45 years old) were at a greater risk of developing more-severe CHIKV disease, including but not limited to persistent CHIKV-associated rheumatic manifestations and even mortality [14]. Increased age has been associated with immune senescence with a decrease in the ability of the immune system to respond to pathogens. An aged CHIKV NHP model was developed in aged (>17 years old) rhesus macaques by Messoudi et al and presented in a study comparing viremia, clinical symptoms, and the CHIKV-specific immune response between CHIKV-infected adult (6–13 years old) and aged (>17 years old) rhesus macaques [43]. In general, both innate and adaptive immune responses were delayed and blunted in aged animals as compared to adult animals; this correlated with CHIKV persistence in aged animals [43]. This initial study, using the aged NHP model of CHIKV infection, identified key differences in the way that aged animals respond to

CHIKV infection as compared to adult animals and suggests that a decline in the immune response, as a result of age, could be a factor leading to increased CHIKV disease severity. Future studies using this model will further delineate the role that age plays in CHIKV immunity and disease severity.

Pregnant NHP Model of CHIK Infection

Intrauterine CHIKV infections were first reported during the outbreak in the Indian Ocean region [49]. It is unknown whether this newly reported mode of transmission is the result of increased case reporting during the outbreak or potentially a result of increased pathogenesis and/or changes in tissue tropism of the virus [49–51]. These unanswered questions led to the development of a pregnant rhesus macaque model of CHIKV infection. In this model, rhesus macaques aged 7–15 years at gestational days 121–132 were infected with CHIKV [42]. Viremia level peaked at 2–3 days after infection, and pregnant animals developed fever and changes in blood cell counts correlating with peak viremia levels. In addition, a limited number of animals developed joint swelling in the wrist and/or ankle, and persistent viral RNA was detectable in the spleen and lymph nodes of pregnant rhesus macaques 21 days after infection. Despite development of viremia and CHIKV dissemination in maternal tissues, the fetal condition remained unaffected, as monitored by fetal heart rate. Infectious CHIKV or viral RNA was not detected in fetal tissues or in the placenta. There were also no histological changes to fetal tissues as a result of CHIKV infection. The lack of CHIKV infection in fetal tissues confirms that prepartum CHIKV did not occur. These data are consistent with studies of CHIKV infection during pregnancy in humans, which demonstrated that intrauterine transmission of CHIKV is very rare during early pregnancy but is more common if mothers are viremic in the week preceding delivery [42, 50]. This model can be used to study not only mother-to-child transmission of CHIKV, but also how CHIKV infections might differ depending on host conditions. A better understanding of CHIKV-host interactions is important to the process of studying chikungunya pathogenesis and the development of new treatment options that can work for a broad spectrum of individuals.

NHPs as Preclinical Models for Testing CHIKV Vaccines and Immunotherapeutics

Live-attenuated, CHIKV/IRES, DNA, virus-like particle, formalin-inactivated, and UV-inactivated formulations of CHIKV vaccines have all been tested in NHP CHIKV models. Similarities in CHIKV infection outcome, physiology, and immune response between humans and NHPs make them ideal models to test potential vaccines and antiviral therapeutics. Cynomolgus macaques may represent an ideal model to assess the safety of live-attenuated virus vaccines. Multiple parameters have been measured during NHP CHIKV infection to assess the effectiveness of potential vaccines and immunotherapeutics at hindering the development of CHIKV infections. These include quantification of virus in circulation (ie, viremia), fever and hypothermia,

rise in heart rate, and virus dissemination [46, 47, 52]. Antibody therapeutics against CHIKV have been tested in a NHP model as a postexposure treatment option for CHIKV infection [47]. Anti-CHIKV mAb treatment blocked viremia and decreased viral RNA loads in tissue sites distal to the site of CHIKV infection. In the arm muscles and axillary lymph nodes, no difference in viral RNA loads was detected between the groups [47]. These differences, or the lack thereof, provided key information about the effectiveness of the mAbs to control virus dissemination and how CHIKV disseminates through the body during a typical CHIKV infection. In the future, other NHP models, such as aged and pregnant rhesus macaque models, could also be used in preclinical trials to assess the effectiveness of potential vaccines and immunotherapeutics under unique host conditions.

SUMMARY

CHIKV is a mosquito-transmitted alphavirus that causes debilitating joint and muscle pain. The polyarthralgia and myalgia can last for months to years after acute infection, significantly influencing the quality of life of those affected. CHIKV historically has caused repeated outbreaks in sub-Saharan Africa and Southeast Asia, and the virus recently emerged in the western hemisphere. Despite the spread of CHIKV to 5 continents and millions of CHIKV disease cases, many aspects of chikungunya pathogenesis are still unknown. Thus, animal models to investigate mechanisms of CHIKV replication and pathogenesis, to define immunological responses to CHIKV infection, and to test novel vaccines and antiviral therapeutics for efficacy against CHIKV infection are indispensable to improve our understanding of this virus and develop treatments that have the potential to improve human health.

Notes

References

1. Dupuis-Maguiraga L, Noret M, Brun S, Le Grand R, Gras G, Roques P. Chikungunya disease: infection-associated markers from the acute to the chronic phase of arbovirus-induced arthralgia. PLoS Negl Trop Dis 2012; 6:e1446.
2. Schilte C, Staikowsky F, Staikovsky F, et al. Chikungunya virus-associated long-term arthralgia: a 36-month prospective longitudinal study. PLoS Negl Trop Dis 2013; 7:e2137.
3. Gerardin P, Fianu A, Malvy D, et al. Perceived morbidity and community burden after a chikungunya outbreak: the TELECHIK survey, a population-based cohort study. BMC Med 2011; 9:5.
4. Couturier E, Guillemin F, Mura M, et al. Impaired quality of life after chikungunya virus infection: a 2-year follow-up study. Rheumatology (Oxford) 2012; 51:1315–22.
5. Marimoutou C, Ferraro J, Javelle E, Deparis X, Simon F. Chikungunya infection: self-reported rheumatic morbidity and impaired quality of life persist 6 years later. Clin Microbiol Infect 2015; 21:688–93.
6. Leparc-Goffart I, Nougairede A, Cassadou S, Prat C, de Lamballerie X. Chikungunya in the Americas. Lancet 2014; 383:514.
7. Weaver SC, Forrester NL. Chikungunya: evolutionary history and recent epidemic spread. Antiviral Res 2015; 120:32–9.
8. Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol 2007; 88:2363–77.
9. Suhrbier A, Jaffar-Bandjee MC, Gasque P. Arthritogenic alphaviruses–an overview. Nat Rev Rheumatol 2012; 8:420–9.
10. Borgherini G, Poubeau P, Staikowsky F, et al. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. Clin Infect Dis 2007; 44:1401–7.
11. Economopoulou A, Dominguez M, Helynck B, et al. Atypical chikungunya virus infections: clinical manifestations, mortality, and risk factors for severe disease during the 2005–2006 outbreak on Reunion. Epidemiol Infect 2009; 137:534–41.
12. Gerardin P, Couderc T, Bintner M, et al. Chikungunya virus-associated encephalitis: a cohort study on La Reunion Island, 2005–2009. Neurology 2015; 86:94–102.
13. Josseran L, Paquet C, Zehgnoun A, et al. Chikungunya disease outbreak, Reunion Island. Emerg Infect Dis 2006; 12:1994–5.
14. Hoarau JJ, Jaffar Bandjee MC, Krejbich Trotot P, et al. Persistent chronic inflammation and infection by chikungunya arthritogenic alphavirus in spite of a robust host immune response. J Immunol 2010; 184:5914–27.
15. Sissoko D, Malvy D, Ezzedine K, et al. Post-epidemic chikungunya disease on Reunion Island: course of rheumatic manifestations and associated factors over a 15-month period. PLoS Negl Trop Dis 2009; 3:e389.
16. Chow A, Her Z, Ong EK, et al. Persistent arthralgia induced by chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor. J Infect Dis 2011; 203:149–57.
17. Ozden S, Huerre M, Riviere JP, et al. Human muscle satellite cells as targets of chikungunya virus infection. PLoS One 2007; 2:e527.
18. Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg (London) 1956; 54:177–91.
19. Couderc T, Chretien F, Schilte C, et al. A mouse model for chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. PLoS Pathog 2008; 4:e29.
20. Werneke SW, Schilte C, Rohatgi A, et al. ISG15 is critical in the control of chikungunya virus infection independent of UbE1L mediated conjugation. PLoS Pathog 2011; 7:e1002322.
21. Levitt NH, Ramsburg HH, Hasty SE, Repik PM, Cole FE Jr, Lupton HW. Development of an attenuated strain of chikungunya virus for use in vaccine production. Vaccine 1986; 4:157–62.
22. Rudd PA, Wilson J, Gardner J, et al. Interferon response factors 3 and 7 protect against chikungunya virus hemorrhagic fever and shock. J Virol 2012; 86:9888–98.
23. Schilte C, Buckwalter MR, Laird ME, Diamond MS, Schwartz O, Albert ML. Cutting edge: independent roles for IRF-3 and IRF-7 in hematopoietic and nonhematopoietic cells during host response to chikungunya infection. J Immunol 2012; 188:2967–71.
24. Gardner CL, Burke CW, Higgs ST, Klimstra WB, Ryman KD. 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 2012; 425:103–12.
25. Plante K, Wang E, Partidos CD, et al. Novel chikungunya vaccine candidate with an IRES-based attenuation and host range alteration mechanism. PLoS Pathog 2011; 7:28.
26. Pal P, Dowd KA, Brien JD, et al. Development of a highly protective combination monoclonal antibody therapy against chikungunya virus. PLoS Pathog 2013; 9:e1003312.
27. Morrison TE, Oko L, Montgomery SA, et al. A mouse model of chikungunya virus-induced musculoskeletal inflammatory disease: evidence of arthritis, tenosynovitis, myositis, and persistence. Am J Pathol 2011; 178:32–40.
28. Gardner J, Anraku I, Le TT, et al. Chikungunya virus arthritis in adult wild-type mice. J Virol 2010; 84:8021–32.
29. Mahauad-Fernandez WD, Jones PH, Okeoma CM. Critical role for bone marrow stromal antigen 2 in acute chikungunya virus infection. J Gen Virol 2014; 95:2450–61.
30. Hawman DW, Stoermer KA, Montgomery SA, et al. Chronic joint disease caused by persistent chikungunya virus infection is controlled by the adaptive immune response. J Virol 2013; 87:13878–88.
31. Poo YS, Rudd PA, Gardner J, et al. Multiple immune factors are involved in controlling acute and chronic chikungunya virus infection. PLoS Negl Trop Dis 2014; 8:e3354.
32. Muthumani K, Lankaraman KM, Laddy DJ, et al. Immunogenicity of novel consensus-based DNA vaccines against chikungunya virus. Vaccine 2008; 26:5128–34.
33. Hallengard D, Kakoulidou M, Lulla A, et al. Novel attenuated Chikungunya vaccine candidates elicit protective immunity in C57BL/6 mice. J Virol 2014; 88:2858–66.
34. Seymour RL, Adams AP, Leal G, Alcorn MD, Weaver SC. A rodent model of chikungunya virus infection in RAG1 -/- mice, with features of persistence, for vaccine safety evaluation. PLoS Negl Trop Dis 2015; 9:e0003800.
35. Teo TH, Lum FM, Claser C, et al. A pathogenic role for CD4+ T cells during chikungunya virus infection in mice. J Immunol 2013; 190:259–69.

36. Lum FM, Teo TH, Lee WW, Kam YW, Rénia L, Ng LF. An essential role of antibodies in the control of chikungunya virus infection. J Immunol 2013; 190:6295–302.

37. Javelle E, Ribera A, Degasne I, Gaüzère BA, Marimoutou C, Simon F. Specific management of post-chikungunya rheumatic disorders: a retrospective study of 159 cases in Reunion Island from 2006–2012. PLoS Negl Trop Dis 2015; 9:e0003603.

38. Threadgill DW, Miller DR, Churchill GA, de Villena FP. The collaborative cross: a recombinant inbred mouse population for the systems genetic era. ILAR J 2011; 52:24–31.

39. Binn LN, Harrison VR, Randall R. Patterns of viremia and antibody observed in rhesus monkeys inoculated with chikungunya and other serologically related group A arboviruses. Am J Trop Med Hyg 1967; 16:782–5.

40. Paul SD, Singh KR. Experimental infection of Macaca radiata with chikungunya virus and transmission of virus by mosquitoes. Indian J Med Res 1968; 56:802–11.

41. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J Clin Invest 2010; 120:894–906.

42. Chen CI, Clark DC, Pesavento P, et al. Comparative pathogenesis of epidemic and enzootic chikungunya viruses in a pregnant Rhesus macaque model. Am J Trop Med Hyg 2010; 83:1249–58.

43. Messaoudi I, Vomaske J, Totonchy T, et al. Chikungunya virus infection results in higher and persistent viral replication in aged rhesus macaques due to defects in anti-viral immunity. PLoS Negl Trop Dis 2013; 7:e2343.

44. Kam YW, Lee WW, Simarmata D, et al. Unique epitopes recognized by antibodies induced in chikungunya virus-infected non-human primates: implications for the study of immunopathology and vaccine development. PLoS One 2014; 9:e95647.

45. Kam YW, Pok KY, Eng KE, et al. Sero-prevalence and cross-reactivity of chikungunya virus specific anti-E2EP3 antibodies in arbovirus-infected patients. PLoS Negl Trop Dis 2015; 9:e3445.

46. Akahata W, Yang ZY, Andersen H, et al. A virus-like particle vaccine for epidemic chikungunya virus protects nonhuman primates against infection. Nat Med 2010; 16:334–8.

47. Pal P, Fox JM, Hawman DW, et al. Chikungunya viruses that escape monoclonal antibody therapy are clinically attenuated, stable, and not purified in mosquitoes. J Virol 2014; 88:8213–26.

48. Broeckel R, Haese N, Messaoudi I, Streblow DN. Nonhuman primate models of chikungunya virus infection and disease (CHIKV NHP Model). Pathogens 2015; 4:662–81.

49. Robillard PY, Boumahni B, Gerardin P, et al. [Vertical maternal fetal transmission of the chikungunya virus. Ten cases among 84 pregnant women]. Presse Med 2006; 35:785–8.

50. Gerardin P, Barau G, Michault A, et al. Multidisciplinary prospective study of mother-to-child chikungunya virus infections on the island of La Reunion. PLoS Med 2008; 5:e60.

51. Touret Y, Randrianaivo H, Michault A, et al. [Early maternal-fetal transmission of the chikungunya virus]. Presse Med 2006; 35:1656–8.

52. Roy CJ, Adams AP, Wang E, et al. Chikungunya vaccine candidate is highly attenuated and protects nonhuman primates against telemetrically monitored disease following a single dose. J Infect Dis 2014; 209:1891–9.