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Review

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Chikungunya virus: an update on the biology and pathogenesis of this emerging pathogen



Felicity J Burt, Weiqiang Chen, Jonathan J Miner, Deborah J Lenschow, Andres Merits, Esther Schnettler, Alain Kohl, Penny A Rudd, Adam Taylor, Lara J Herrero, Ali Zaid, Lisa F P Ng, Suresh Mahalingam

Re-emergence of chikungunya virus, a mosquito-transmitted pathogen, is of serious public health concern. In the past 15 years, after decades of infrequent, sporadic outbreaks, the virus has caused major epidemic outbreaks in Africa, Asia, the Indian Ocean, and more recently the Caribbean and the Americas. Chikungunya virus is mainly transmitted by *Aedes aegypti* mosquitoes in tropical and subtropical regions, but the potential exists for further spread because of genetic adaptation of the virus to *Aedes albopictus*, a species that thrives in temperate regions. Chikungunya virus represents a substantial health burden to affected populations, with symptoms that include severe joint and muscle pain, rashes, and fever, as well as prolonged periods of disability in some patients. The inflammatory response coincides with raised levels of immune mediators and infiltration of immune cells into infected joints and surrounding tissues. Animal models have provided insights into disease pathology and immune responses. Although host innate and adaptive responses play a role in viral clearance and protection, they can also contribute to virus-induced immune pathology. Understanding the mechanisms of host immune responses is essential for the development of treatments and vaccines. Inhibitory compounds targeting key inflammatory pathways, as well as attenuated virus vaccines, have shown some success in animal models, including an attenuated vaccine strain based on an isolate from La Reunion incorporating an internal ribosome entry sequence that prevents the virus from infecting mosquitoes and a vaccine based on a virus-like particle based expressing envelope proteins. However, immune correlates of protection, as well as the safety of prophylactic and therapeutic candidates, are important to consider for their application in chikungunya infections. In this Review, we provide an update on chikungunya virus with regard to its epidemiology, molecular virology, virus-host interactions, immunological responses, animal models, and potential antiviral therapies and vaccines.

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National Health Laboratory Services, Universitas and Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa (Prof F J Burt PhD); Institute for Glycomics, Griffith University, Gold Coast, QLD, Australia (W Chen PhD, P A Rudd PhD, A Taylor PhD, L J Herrero PhD, A Zaid PhD, Prof S Mahalingam PhD); Department of Internal Medicine, Washington University School of Medicine, St Louis, MO, USA (J J Miner MD, D J Lenschow MD); Institute of Technology, University of Tartu, Tartu, Estonia (Prof A Merits PhD); MRC-University of Glasgow Centre for Virus Research, Glasgow, Scotland, UK (E Schnettler PhD, A Kohl PhD); Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR), Biopolis, Singapore (Prof L F P Ng PhD); and Institute of Infection and Global Health, University of Liverpool, Liverpool, UK (Prof L F P Ng)

Correspondence to: Dr Felicity J Burt, Department of Medical Microbiology and Virology, National Health Laboratory Services, Universitas and Faculty of Health Sciences, University of the Free State, Bloemfontein 9300, South Africa burtFJ@ufs.ac.za

Introduction

Chikungunya fever is a debilitating arthritic disease caused by chikungunya virus. The virus was first identified in 1952–53 during an outbreak that occurred in the Makonde Plateau in the southern region of Tanzania.¹ The name “chikungunya” is derived from a Swahili or Makonde word meaning “that which bends up”, and refers to the bending posture of individuals infected with the virus.¹ The virus belongs to the genus alphavirus of the Togaviridae family and, similar to other arthritogenic alphaviruses, its infection is commonly characterised by acute fever that progresses to severe, persistent arthralgia in the chronic stage of disease.² The disease is usually self limiting, but in some patients debilitating joint pain can persist for years. The increased frequency of outbreaks in the past 15 years appears to be associated with a higher incidence of more severe forms of the disease than previously described, with reports of cases of neurological involvement, fulminant hepatitis, and neonatal encephalopathy.^{3–6}

The virus usually circulates in a sylvatic cycle between non-human primates or mammalian reservoir hosts and *Aedes* species mosquitoes. During urban epidemics, chikungunya virus can be transmitted to human hosts through infectious bites by *Aedes* spp mosquitoes.⁷ Since 2000, the incidence of large outbreaks has increased with spread of the virus to previously non-endemic regions; moreover, concomitant evidence of genetic adaptation of chikungunya virus to *Aedes albopictus* has implications for the spread of the virus to non-endemic regions.^{8,9} *Aedes aegypti* is

found in tropical and subtropical regions, whereas *A. albopictus* has a wider distribution and is found in temperate regions. Outbreaks of disease caused by an East Central South African (ECSA) genotype have been reported in Europe.^{10–12} Although these outbreaks have been sporadic without extensive spread, the presence of *A. albopictus* raises concerns about the potential for the virus to establish endemicity in southern Europe.^{10,11}

In contrast to the sporadic outbreaks occurring in Europe, the virus was identified in the western hemisphere in October, 2013, when an outbreak caused by the Asian genotype was identified on Saint Martin Island. From there, the virus has rapidly spread with autochthonous transmission confirmed in multiple countries and territories in the Caribbean and the Americas.^{13–16} *A. albopictus*, present in southern and eastern regions of the USA, is a potential vector for further spread of this virus and establishment of endemic regions in the USA. The current outbreaks of arboviruses such as chikungunya virus, Zika virus, and yellow fever virus highlight the importance of understanding the epidemiological factors contributing to these epidemics, promoting pathogenicity, and affecting control measures.

Chikungunya virus continues to cause large epidemics worldwide, with no specific treatment or vaccine currently available to prevent infection. In this Review, we provide an update on chikungunya virus with regard to its epidemiology, molecular virology, virus-host interactions, immunological responses, animal models of disease, and potential antiviral therapies and vaccines.

Genome organisation and molecular structure of chikungunya virus

Chikungunya virus has an approximately 12 kb positive-sense RNA genome that encodes four non-structural proteins (nsP1–4), with five structural proteins (C, E3, E2, 6K, and E1) expressed from subgenomic RNA synthesised in infected cells. The genome has a short 5′ untranslated region and a longer 3′ untranslated region comprising stem-loop structures and direct repeats that are thought to be associated with adaptation of the virus to mosquito hosts.¹⁷ The genome is packed into virions that are similar to those of other alphaviruses. The cellular receptors for chikungunya virus remain unknown. Chikungunya virions are internalised by clathrin-mediated endocytosis, but the available evidence also suggests that the entry pathway might be cell-type specific or that multiple pathways are used.¹⁸

Replication of chikungunya virus RNA is preceded by its translation, which results in the production of non-structural (ns) polyproteins P123 and P1234. The non-structural polyproteins are processed into mature non-structural proteins by the protease activity of the nsP2 region.¹⁹ Mature nsP1 plays a role in viral replication as described for other alphaviruses. In addition to having enzymatic activities, including NTPase and RNA triphosphatase activity,²⁰ RNA helicase activity,²¹ and protease activity,¹⁹ nsP2 can counteract host cellular antiviral responses through multiple mechanisms. These mechanisms include general transcriptional shutdown by degradation of the Rpb1 subunit of host cellular RNA polymerase II,²² as well as more specific mechanisms including interference with JAK/STAT signalling,^{23,24} the unfolded protein response,²⁵ and autophagy.²⁶ The three-dimensional structure of the N-terminal (macro) domain of nsP3 has been resolved and shown to bind negatively charged polymers, including RNA.²⁷ Increasing evidence also suggests that the intrinsically unstructured C-terminal region of nsP3 acts as a binding platform for numerous host cellular proteins, including G3BP proteins^{24,28} and amphiphysins,²⁹ and that these interactions are important for virus infection. Despite the absence of direct evidence, nsP4 of chikungunya virus clearly appears to be an RNA-dependent-RNA polymerase.

Considerable attention has been dedicated to the identification of host proteins that interact with the non-structural proteins of chikungunya virus.^{30–33} Cellular proteins also interact with viral RNA.³⁴ In most cases the functional importance of these interactions remains unknown. However, some interactions have a positive effect on virus replication, whereas others mediate antiviral effects. Some interacting proteins might have both proviral and antiviral effects. For example, the interaction of nsP3 of alphaviruses with G3BP proteins has an antiviral effect by preventing the formation of stress granules.³⁵ A proviral function for G3BP proteins has been shown, with depletion of these proteins resulting in inhibition of early replication.³⁶

One possible role of G3BP proteins is to facilitate switching from non-structural protein translation to replication of genomic RNA;³⁶ however, the exact details of RNA replication of chikungunya virus have not been studied. By analogy with Semliki Forest virus and Sindbis virus, replication first generates negative-strand RNA, probably existing exclusively in duplex form with the positive-strand genome, and then generates numerous positive—genomic and subgenomic—RNAs.³⁷ Genomic RNA contains a packaging signal that, unlike most of the alphaviruses, is located in the region encoding nsP2.³⁸

Structural proteins, which are essential for virion formation, are translated from subgenomic RNA. Chikungunya infection shuts down translation of cellular mRNAs, and subgenomic RNA remains the only actively translated mRNA in the cell.³⁹ Unlike Semliki Forest virus and Sindbis virus, subgenomic RNA of chikungunya virus does not have a stable stem-loop structure, the so-called capsid enhancer, at its 5′ region (Merits A, unpublished). Thus, how active translation of chikungunya virus RNA is maintained remains unclear. The first structural protein, the capsid protein, is not only responsible for nucleocapsid formation but also possesses nuclear export and import signals allowing entry to and exit from the nucleus.⁴⁰ The glycoprotein part of the structural polyprotein is translated by membrane-associated ribosomes and is processed and post-translationally modified by cellular enzymes. The assembly and budding of chikungunya virions takes place on the plasma membrane of infected vertebrate cells. This process is sensitive to antiviral effects of the cellular protein tetherin,⁴¹ which are counteracted by nsP1.⁴² In polarised cells, the release of chikungunya virions occurs at the apical domain of cells; data obtained with different inhibitors suggest that the N-glycans of chikungunya virus envelope glycoproteins could serve as apical sorting signals.⁴³

Epidemiology and evolution of chikungunya virus

Chikungunya virus was first isolated in 1952 in Tanzania.¹ Before 2000, outbreaks of chikungunya virus occurred sporadically, with reports of naturally acquired human infection from Angola,⁴⁴ Benin,⁴⁵ Burundi,⁴⁶ Cameroon,^{47,48} the Central African Republic,⁴⁹ Democratic Republic of the Congo,⁵⁰ Gabon,⁵¹ Guinea,⁵² Kenya,⁵³ Liberia,⁵⁴ Madagascar,⁵⁵ Malawi,⁵⁶ Nigeria,⁵⁷ Uganda,⁵⁸ Senegal,⁵⁹ Sierra Leone,⁶⁰ southern Africa,^{3,54,60} Sudan,⁶¹ and Tanzania.¹

The virus is believed to have originated in Africa, with subsequent spread to Asian countries probably occurring via shipping. The earliest confirmation of disease caused by chikungunya virus in Asia was reported from the Philippines in 1954. Outbreaks have subsequently been reported in southern and southeast Asia, including Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Laos, Malaysia, Maldives, Burma/Myanmar, Pakistan, Saudi Arabia, Singapore, Sri Lanka, Taiwan, Thailand, Timor, Vietnam, and Yemen.^{62–68} Genetic analyses of

strains have identified three distinct lineages of chikungunya virus: the West African lineage, the ECSA lineage, and the Asian lineage^{9,69,70} derived from the ECSA virus.

Since 2000 the virus has been re-emerging, causing several outbreaks of more severe forms of the disease than previously reported.^{50,71} In 2004 an epidemic strain of the ECSA lineage emerged and spread from coastal towns of Kenya to the Indian Ocean islands, causing an outbreak of unprecedented magnitude.^{72–76} Concurrently, re-emergence of the virus was reported in India, after an absence of 32 years, affecting 13 different states during 2005–06.^{77,78}

The epidemic strain of chikungunya virus circulating during the outbreak in the Indian Ocean islands, referred to as the Indian Ocean Lineage, was likely to have been transmitted primarily by *A albopictus*, the predominant mosquito in the region at that time. In later stages of the outbreak, the Indian Ocean Lineage appeared to acquire a mutation in the envelope glycoprotein (the E1-A226V mutation). This mutation contributed to a gain of fitness adaptation for dissemination by *A albopictus*, and the ability of the virus to adapt and replicate in this vector probably contributed to the magnitude of the outbreak.⁸ Interestingly, although the E1-A226V mutation improves the ability of the virus to infect and replicate in *A albopictus*, it has no effect on infection of *A aegypti*.^{8,79–81}

Although the outbreaks in the Indian Ocean islands and India have been attributed to an ECSA strain with very high nucleotide similarity between isolates from India and from the Indian Ocean islands, the mutation that is putatively associated with adaptation to *A albopictus*

was not detected in isolates circulating in India in 2005. Evidence from a mosquito isolate collected in Maharashtra in 2000 suggests that a switch in the circulating chikungunya virus genotype, from Asian to African, occurred in India before the 2004 outbreak in the Indian Ocean islands and before the 2005 outbreak in India⁷⁷ (figure 1).

In the western hemisphere chikungunya virus was initially identified on Saint Martin Island in October, 2013, and from there the virus rapidly spread to countries and territories in the Americas.^{14,15} From the onset of the outbreak to early August, 2016, autochthonous transmission of the virus has been confirmed in 48 countries or territories in the Caribbean, Central America, South America, and North America, with more than 1 million suspected cases. The spread and establishment of the virus in new endemic regions is likely to be dependent on the availability of competent vectors.

Genetic characterisation showed that the strain circulating in the Caribbean and Americas is an Asian strain, closely resembling the strains circulating in the Philippines (2013),⁸² China (2012), and Yap (2013) in southeast Asia^{14,83} (figure 1). In addition, ECSA strains identified in Brazil in 2014 resemble the strains circulating in Angola, with evidence of infection occurring in local residents with no travel history.⁸⁴

Vectors and arbovirus-vector interactions

Innate immune responses of the mosquito vector are important in controlling the replication and transmission of chikungunya virus. Immune pathways in *A aegypti* mosquitoes—such as Toll, JAK-STAT, and Imd—have

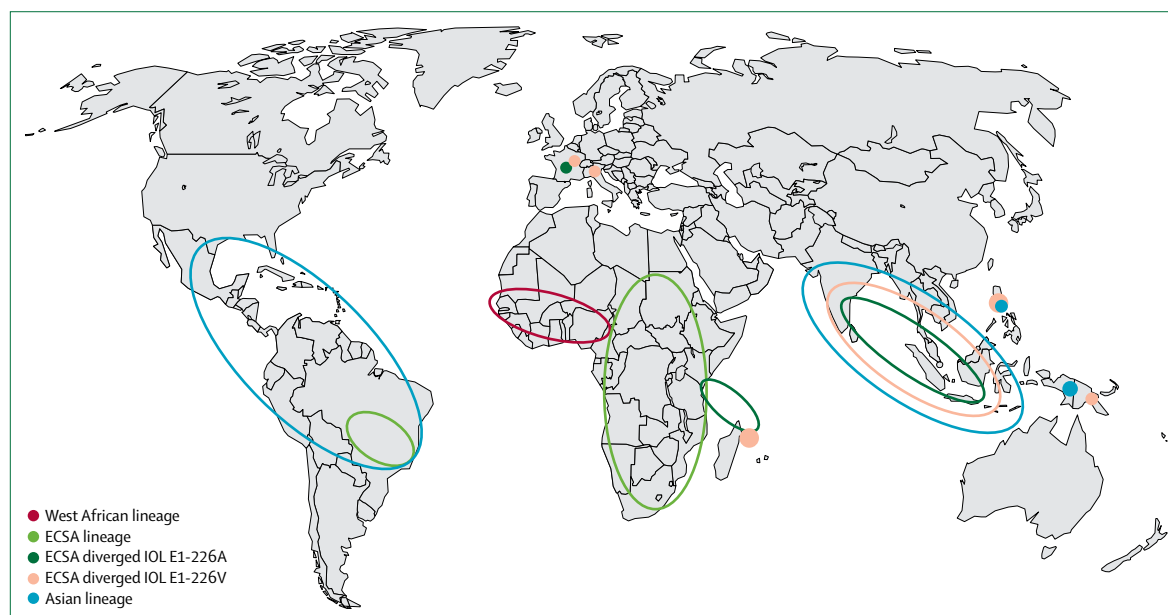


Figure 1: Distribution of chikungunya virus

The figure shows the spread and divergence of the East Central South African (ECSA) lineage to the Indian Ocean islands (IOLs) and Asia,^{9,72,73} the spread of the Asian lineage to the Americas in 2013,⁸⁶ and the spread of the ECSA lineage to Brazil in 2014.⁸⁷ Autochthonous cases identified in Europe and their genetic lineage are shown.^{10–12}

antiviral roles.⁸⁵ Although chikungunya virus infection can repress induction of the Toll pathway in vitro, no antiviral effect has been observed for the Toll, JAK-STAT, and Imd pathways in *A aegypti* cells.⁸⁶ RNA interference (RNAi) is believed to be the main mosquito antiviral response. RNAi can be categorised into several distinct pathways, on the basis of the small RNAs involved: 21-nucleotide small interfering RNA (siRNA), 21–22-nucleotide microRNA (miRNA), and 24–30-nucleotide piwi-interacting RNA (piRNA) pathways.⁸⁷ Each pathway has distinct roles in cellular processes and arbovirus-host interactions. The antiviral role of the siRNA pathway has been established for mosquito-borne arboviruses, and research⁸⁸ suggests a similar activity for the piRNA pathway in *Aedes* spp mosquitoes. Chikungunya-specific siRNAs and piRNAs have been reported in infected *Aedes* spp mosquitoes and cell lines, indicating that these RNAs have antiviral activity.⁸⁸ This is supported by the ability of the siRNA pathway, specifically the protein Argonaute-2, to limit the spread of chikungunya infection,⁸⁶ and the reported increase in virus production and pathogenicity in mosquitoes infected with chikungunya virus and expressing suppressor proteins that interfere with the siRNA pathway.⁸⁸ In vertebrates and invertebrates, the miRNA pathway is important in regulating gene expression on a post-transcriptional level;⁸⁹ however, little is known about the interactions of arboviruses with the mosquito miRNA pathway. Changes in miRNA expression following chikungunya infection have been reported in *A albopictus* cells, with upregulation and downregulation observed. Target predictions identified the involvement of mosquito mRNAs in many pathways, but more research is needed to validate these targets. Combinational analysis of differentially expressed miRNAs in mosquito cells infected with chikungunya virus versus those infected with *Plasmodium* spp showed that most of these miRNAs are regulated in a pathogen-specific manner.⁹⁰ Similarly, differential expression of miRNAs has been reported in the saliva of *A aegypti* and *A albopictus* mosquitoes infected with chikungunya virus. Although none of the targets is yet known, inhibition studies in mosquito and mammalian cells showed a reduction in chikungunya virus production, suggesting an interaction between the virus and the miRNA pathway.⁹¹ The presence of these miRNAs in mosquito saliva and in mammalian cells could point to an effect in the vertebrate host.

Clinical presentation

Patients with acute chikungunya virus infection usually have an abrupt onset of high fever (>39°C), severe arthralgia and myalgia, and an erythematous, maculopapular rash, which can range in severity from a mild, localised rash to an extensive rash involving more than 90% of the skin⁹² (figure 2). The abrupt onset of these symptoms occurs after a mean incubation period of 3 days. The rash and fever usually resolve within a few days and are occasionally followed by palmoplantar

desquamation.⁵⁹ Less common symptoms include ocular manifestations such as conjunctivitis, uveitis, episcleritis, and retinitis.⁹³ About 15% of individuals infected with chikungunya virus are asymptomatic.⁹² Most patients have joint pain and swelling with severe morning stiffness, consistent with inflammatory arthritis⁹² (figure 2). The joint pain is typically symmetrical and almost any joint can be affected, especially during the acute phase, although the distal extremities are affected more frequently.^{92,94,95} Synovitis or periarticular swelling has been reported in 32–95% of patients, with large joint effusions occurring in 15% of individuals infected with chikungunya.^{96–98} In many patients, chikungunya-related joint pain begins to improve after the first week, although some patients have persistent joint pain, swelling, and morning stiffness. These symptoms can last for up to 3 years.^{99,100}

Death from chikungunya infection is rare and occurs in fewer than one in 1000 individuals.⁴ However, severe infection can present with encephalitis and encephalopathy, myocarditis, hepatitis, and multi-organ failure. Neuroinvasion by chikungunya virus, causing seizures, altered mental status, flaccid paralysis, and even death, occurs infrequently.^{6,101–103} Case reports point to a heightened risk of severe disease in neonates, elderly people aged above 65 years with other underlying medical conditions, and immunosuppressed individuals. Severe neonatal chikungunya infection, including infection resulting from mother-to-child transmission, was documented in the La Reunion epidemic.¹⁰⁴ In neonates born to mothers with viraemia, the prevalence [A: is this correct?] of infection reached 50%. Neuroinvasion was reported in 22 out of 24 neonatal cases. Children with chikungunya-associated encephalopathy have poor long-term neurocognitive outcomes, which can include severe sequelae such as microcephaly and cerebral palsy.¹⁰⁵ In addition to neuroinvasive disease, some neonates develop a haemorrhagic syndrome, necrotising enterocolitis, haemodynamic disorders, ventricular dysfunction, pericarditis, and coronary artery dilatation.^{101,104}

Persistent arthralgia occurs after resolution of the acute phase of infection (7–10 days). Multiple joints are usually affected in the chronic phase, especially the small joints of the hands, feet, ankles, and wrists^{92,94} (figure 2), although larger joints could also be affected. Remarkably, some patients develop bone erosions as a result of chikungunya-induced arthritis.^{106–108} The exact prevalence of bone erosions is yet to be determined, but bone erosions might be a less common phenomenon. Nonetheless, the ability of chikungunya to cause erosive disease distinguishes arthritogenic alphaviruses from other forms of viral or post-viral arthritis.

Persistent joint pain caused by chikungunya virus infection is often debilitating, and the natural course of disease involves gradual improvement until complete resolution of symptoms. In La Reunion, up to 60% of patients had relapsing and remitting arthralgia up to

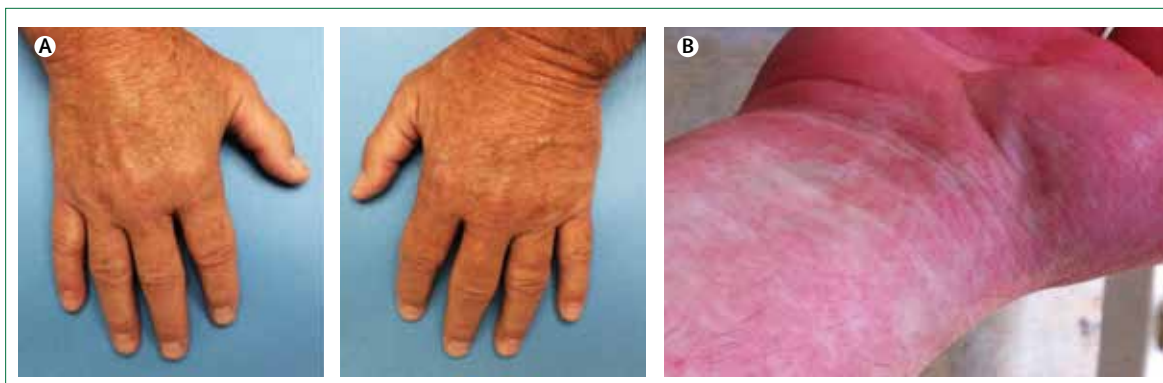


Figure 2: Clinical features of disease in patients with chikungunya virus infection

(A) Maculopapular rash during the acute infectious phase. The rash was distributed over the entire body, resolved after a few days, and was followed by desquamation. The patient was a white woman aged 33 years. The patient developed fever, diffuse arthritis, and an erythematous, maculopapular rash over her entire body. (B) Active symmetric synovitis in the metacarpophalangeal and proximal interphalangeal joints during the chronic phase of chikungunya-associated arthritis.

The patient was a white man aged 57 years. The patient developed high fever, a diffuse maculopapular rash, joint pain, stiffness, and swelling consistent with an acute pattern. Both individuals travelled to Haiti during a chikungunya virus outbreak. Figure reproduced from Miner JJ et al, *Arthritis Rheumatol* 2015; **67**: 1214–20,⁹⁵ by permission of John Wiley & Sons, Inc. [A: panels A and B swapped to match revised text — OK?]

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36 months after being diagnosed with acute disease.¹⁰⁰ Risk factors for the development of long-term arthralgia following chikungunya virus infection include age (>35 years) and the presence of arthralgia in the first 4 months after onset of symptoms.¹⁰⁰ A further complicating diagnostic issue is that persistent chikungunya-induced joint pain can mimic symptoms of rheumatoid arthritis. Similarities between the persistent phase of chikungunya and rheumatoid arthritis could confound the diagnosis, although most patients infected with chikungunya virus have an abrupt onset of arthritis and fever, which would be unusual in adult-onset rheumatoid arthritis. However, acute joint pain caused by chikungunya is not always diagnostically distinguishable from juvenile idiopathic arthritis or systemic-onset juvenile rheumatoid arthritis, which are frequently associated with fever and rash. Although chikungunya-induced joint pain is self limiting, the prolonged and severe nature of the illness can have a major impact on society in terms of morbidity and economic productivity.⁹⁹

Pathogenesis of chikungunya virus

During the early and acute phase of infection, high titres of chikungunya virus are present in the blood, resulting in viraemia that can be detected by real time PCR within the first few days of infection. The resulting inflammatory response coincides with elevation of immune mediators followed by infiltration of immune cells into infected joints and surrounding tissues.

Patients with acute and chronic chikungunya virus infection have high concentrations of circulating cytokines and chemokines.^{109–111} However, considerable variation exists among results from these studies. A meta-analysis¹¹² found raised levels of numerous serum and plasma cytokines in cohorts of patients from different regions of the world. Chikungunya virus infection resulted in raised

concentrations of several pro-inflammatory cytokines (interferon- α , interferon- γ , interleukin-6, and others), anti-inflammatory cytokines (interleukin-1 receptor-a, interleukin-4, and interleukin-10), and other chemokines such as IP-10 and monocyte chemotactic protein 1.¹¹²

The number of circulating activated and effector T cells is increased in patients with persistent chikungunya-induced arthritis,⁹² and studies in mice suggest that T cells play a major role in the pathogenesis of chikungunya-induced arthritis.¹¹³ Patients infected with chikungunya develop a robust antibody response, with IgM concentrations detectable within days of infection and neutralising anti-chikungunya IgG typically measurable in the second week of infection. Antibodies to chikungunya are important for clearance of the infectious virus.¹¹⁴ Neutralising anti-chikungunya IgG persists for at least 21 months¹¹⁵ and probably for years, thereby providing strong antiviral immunity that prevents clinical symptoms in the event of a second infection with chikungunya virus. Potently neutralising human monoclonal antibodies to chikungunya are known to bind the E2 envelope glycoprotein.¹¹⁶

In addition to T and B cells, which are involved in pathogenesis of chikungunya virus, multiple other cell types are likely to play a role during infection. Animal models and in vitro studies have shown that chikungunya virus infects multiple cell types, including dendritic cells, macrophages, synovial fibroblasts, endothelial cells, and myocytes.² Chikungunya virus infects human osteoblasts and causes cytopathic effects,¹¹⁷ which could contribute to the joint pathology and erosive disease. Moreover, greater numbers of natural killer cells have been found in the peripheral blood of patients with persistent chikungunya-induced arthritis than in healthy controls.⁹²

Most studies have focused on the innate immune response during acute chikungunya virus infection. Why a subset of patients develop persistent arthritis is unclear,

and the immune pathways that control or trigger these chronic symptoms remain undefined. At least three hypotheses have been put forward to explain why patients with chikungunya frequently develop chronic arthritis: 1) persistence of the infectious virus; 2) persistence of viral nucleic acids, which could trigger persistent immunopathology; and 3) triggering of persistent immune activation in certain individuals after the infectious virus has been cleared. Although none of these hypotheses has been proven correct, clinical studies and animal models have yielded intriguing results. For example, viral proteins have been detected in macrophages in chikungunya-infected macaques long after acute infection has resolved, suggesting persistence of the infectious virus.¹¹⁸ However, infectious virus has never been cultured from patients after the first week of infection, suggesting that replicating but defective viral genomes—which are unable to produce infectious virus—might persist in the joints of infected individuals. Additional studies are required to distinguish between these intriguing possibilities and to develop effective therapeutics, especially because immunosuppressive medications could be deleterious in the context of a persistent infection.

Insights from non-human primate and mouse models

The first studies of chikungunya virus infection in non-human primates were done in the late 1960s in rhesus macaques (*Macaca mulatta*) and bonnet macaques (*Macaca radiata*).^{119,120} These studies showed the susceptibility of non-human primates to chikungunya virus infection and the transmissibility of chikungunya virus from mosquitoes to non-human primates. In 2010, Labadie and colleagues¹¹⁸ developed a more detailed primate model by intravenously infecting cynomolgus macaques (*Macaca fascicularis*) with a chikungunya virus isolate (LR2006-OPY1) from the La Reunion outbreak. Infected macaques developed clinical signs of chikungunya that closely resembled those seen in individuals with chikungunya.¹¹⁸ During acute infection, high amounts of chikungunya virus RNA were detected in the spleens, lymph nodes, and livers, and comparatively lower amounts detected in joints, muscle, skin, and the CNS. Analysis of the subacute and chronic phases showed that secondary lymphoid organs were infiltrated by macrophages, and chikungunya virus antigens were present in several tissues, including lymphoid organs, meninges, joints, and muscles.¹¹⁸

To examine the potential for vertical transmission of chikungunya virus infection, pregnant non-human primates were inoculated subcutaneously with chikungunya virus from either the ECSA or the Asian lineages.¹²¹ Vertical transmission of chikungunya virus was not seen, suggesting that in cases where neonates are born to mothers with viraemia, transmission of chikungunya from mother to child is likely to occur

during delivery rather than vertically in utero.¹²¹ Age-related immunity has also been investigated in non-human primates, with old animals (aged over 17 years) compared with adult animals (aged 6–13 years).¹²² Findings suggest that immune senescence can affect both innate and adaptive immune responses to chikungunya.

The most widely documented mouse models of acute chikungunya infection involve subcutaneous ventral footpad injection of the virus in either young wild-type C57BL/6 mice (3–4 weeks of age) or adult wild-type C57BL/6 mice (>6 weeks of age).^{113,114,123,124} A study¹²⁴ found a severe reduction in bone volume in the tibial epiphysis of the knee in chikungunya-infected mice. Disruption of the receptor activator of nuclear factor- κ B ligand (RANKL) and osteoprotegerin ratio in infected bone tissue promoted a pro-osteoclastic microenvironment and bone resorption. Moreover, dampening recruitment of monocytes and macrophages to skeletal joints of chikungunya-infected mice with the use of bindarit, a monocyte chemotactic protein inhibitor, reduced osteoclastogenesis and prevented severe bone loss. Interestingly, studies have identified a dual role for CCR2⁺ monocytes and macrophages not only as inducers of footpad swelling and inflammatory pathologies but also in preventing excessive inflammatory pathology and resolving chikungunya-induced inflammation.^{124,125} With this in mind, the absence of CCR2 in chikungunya-infected mice resulted in increased neutrophil infiltration in joints, leading to erosive cartilage damage. Overall, findings from mouse models of acute chikungunya infection have provided new insights into chikungunya disease pathophysiology.

Early mouse models of chikungunya virus used immunologically immature neonates or mice with abrogated type I interferon responses. Since then, the role of the innate immune response in the pathogenesis of chikungunya virus has been examined extensively.^{124,126,127} Musculoskeletal inflammatory disease following chikungunya infection is greatly exacerbated in the absence of responses dependent on STAT1 signalling and type I interferon receptor signalling.¹²⁷ Moreover, RIG-I/MDA-5 signalling via IPS-1, the TLR3/TRIF pathway, and, to a lesser extent, MyD88-dependent signalling are important for interferon- α and interferon- β production in response to chikungunya infection.^{127,128} The contribution of CD4⁺ T cell responses to chikungunya-induced arthritis has also been explored, with MHCII and CD4⁺ knockout mice showing considerable reductions in footpad swelling following infection.^{113,129} Viraemia was controlled in CD4⁺ knockout mice, showing the importance of CD4⁺ T-helper-independent antibody responses in minimising virus replication.¹¹³ Chronic chikungunya infection of *Rag2*^{-/-} mice was replicated in separate studies,^{114,130} in which persistence of chikungunya virus RNA in joint-associated tissues was associated with histopathological evidence of arthritis, synovitis, and tendonitis.¹³⁰ Anti-

chikungunya monoclonal antibody therapy had only 1 tissue-specific efficacy in clearing chikungunya virus from *Rag2^{-/-}* mice and was not effective in preventing persistence of chikungunya infection in the joints.¹³⁰ Together, these findings suggest that adaptive immunity 5 controls the persistence of chikungunya infection, which subsequently leads to the chronic musculoskeletal tissue pathology.

Control strategies: antivirals, vaccines, and antibodies 10

No licensed vaccine or antiviral drug is available against chikungunya virus. Current treatment mainly involves the use of anti-inflammatory drugs for symptomatic relief. During the La Reunion outbreaks, chloroquine 15 (commonly used as an antimalarial drug) was used for clinical treatment of the disease. Although chloroquine has strong anti-chikungunya effects in cell culture, it had no effects on patients.¹³¹ Broad-spectrum antivirals like ribavirin and interferon were effective against 20 chikungunya,¹³² whereas mycophenolic acid was shown to be more potent than ribavirin in controlling chikungunya virus replication in various cellular studies.^{39,133} Further investigation of these drugs will be necessary before they can be used for routine clinical 25 treatment of chikungunya infection.

Most drug discovery studies have relied on cell-based screens with antiviral compounds that mainly target the proteins involved in virus replication.¹³⁴ The polymerase inhibitor favipiravir has been shown to inhibit 30 chikungunya virus in cell culture and in a lethal mouse model.¹³⁵ Although the mode of action remains undefined, modified nucleosides such as 6-azauridine and 3-deaza-adenosine are also active against chikungunya virus replication.³⁹ Chikungunya virus 35 nsP2 is both a helicase and a protease, and attempts have been made to identify known protease inhibitors that could inhibit the virus.¹³⁶

Viral replicase inhibitors such as betulins, trigocherrins, and trigocherriolides, as well as 12-O-tetradecanoylphorbol 40 13-acetate, a potent tumour inhibitor, are effective against chikungunya virus through mechanisms not yet determined.¹³⁷⁻¹³⁹ Compounds that interfere with chikungunya virus replication by targeting host processes have also been reported. These include inhibition of the 45 heat shock protein HSP90 and inhibition of kinases and other cellular signalling pathways.^{33,140} The current challenge for antiviral compounds against chikungunya virus is that, although a number of hits have been identified, further research is needed to determine which 50 compound will make it to the clinic.

The first chikungunya vaccines were developed in the 1960s with formalin-inactivated virus preparations and attenuated strains, but none was successful.^{141,142} Subsequently, an attenuated vaccine candidate, TSI-GSD-218 55 (also known as 181/clone25), was developed by the US Army Medical Research Institute on the basis of a

Search strategy and selection criteria

We searched the electronic database PubMed using the terms “chikungunya” or “alphavirus” for manuscripts published from Jan 1, 2000 to Aug 31, 2016. We included selected publications that provided recent information on chikungunya virus with regard to its epidemiology, molecular virology, virus-host interactions, immunological responses, animal models, and potential antiviral therapies and vaccines.

clinical isolate originating from Thailand in 1962.^{143,144} However, development of this vaccine was discontinued because of a scarcity of funds and insufficient market interest.¹⁴⁴ Another attenuated vaccine was developed with the La Reunion isolate to contain an internal ribosomal entry site element between the non-structural and the structural genes. This vaccine produced high amounts of neutralising antibodies in mice and protected the animals from chikungunya virus.^{145,146} Cross-protective immunity was also observed against o'nyong-nyong virus.¹⁴⁷ In non-human primate models, this vaccine prevented viraemia following challenge with chikungunya virus.¹⁴⁸ Other attenuated chikungunya vaccines with large deletions in either the nsP3 or 6K genes were shown to generate robust immune responses after a single immunisation and to fully protect mice from a high-dose challenge with chikungunya virus.¹⁴⁹

Virus-like particles have also been investigated as candidate vaccines.¹⁵⁰⁻¹⁵² Virus-like particles encoding the capsid and envelope glycoproteins were immunogenic in mice and non-human primates, inducing high concentrations of protective neutralising antibodies.^{150,153} Similarly, a measles-virus-based vaccine, which also expressed chikungunya virus-like particles, protected susceptible mice from lethal chikungunya virus challenge.¹⁵⁴ Both of these virus-like particle vaccines have been used in clinical trials^{155,156} in which vaccinated healthy adults produced neutralising antibodies to chikungunya virus with no reports of adverse events.

Various other strategies have been developed over the years, including with non-alphavirus vectors for expression of the structural genes of chikungunya virus, which completely protected mice from viraemia and arthritis after challenge with the La Reunion and Asian isolates.¹⁵⁷ Other candidates successfully tested in mice include the vaccine based on a chimeric vesicular stomatitis virus in which the glycoprotein (G) gene of vesicular stomatitis virus was replaced by the entire region encoding the chikungunya virus structural polyprotein,¹⁵⁸ and a recombinant poxvirus-chikungunya vaccine candidate based on the modified vaccinia virus Ankara strain expressing the structural genes of chikungunya virus.¹⁵⁹

DNA-based vaccines expressing chikungunya virus envelope proteins E3, E2, and E1,¹⁶⁰ and the capsid protein,¹⁶¹ have been investigated. Use of the

chikungunya virus nsP2 gene as an adjuvant led to improved immune responses and better protection of the animals following challenge.¹⁶² Subunit vaccines have also been investigated with bacterially produced recombinant E2 and E1 protein antigens delivered in combination with a number of different adjuvants. These vaccines induced the production of high amounts of neutralising antibodies.^{163,164}

Passive immunisation in various animal models has also been shown, including with human polyvalent antibodies¹⁶⁵ purified polyclonal antibodies from non-human primates immunised with chikungunya virus-like particle vaccines,¹⁵⁰ and human neutralising monoclonal antibodies directed against E2 and E1 glycoproteins.^{166–168} In all of these cases, the antibodies exhibited strong neutralising activities and protected susceptible adult *Ifnar*^{-/-} mice and neonatal C57BL/6 mice from infection. Passive immunisation could therefore constitute an effective medical intervention for individuals who have been exposed to chikungunya virus and are at risk of developing severe disease.

Nonetheless, as most vaccine candidates and biologics have so far been tested only in mice, many remain in the preclinical phase; concerted efforts will be required to advance these compounds into clinical trials. The immune correlates of protection, plus the safety and stability of these prophylactic and therapeutic candidates, are important aspects to consider in the quest to control and treat chikungunya infections.

Conclusion

Novel insights into the mechanisms of the disease pertaining to joint and bone damage, as well as the dynamics of the protective immune responses, are contributing to the development of therapeutics against chikungunya. Inhibitory compounds targeting key inflammatory pathways, as well as attenuated virus vaccines, have shown promising results in animal models. However, further characterisation is needed of key pathways in host-pathogen interactions and the inflammatory cascades that result in disease to help design better, more targeted therapies.

Contributors

FJB, WC, JJM, DJL, AM, ES, AK, PAR, AT, LJH, AZ, LFPN, and SM drafted sections of the Review on the basis of their own literature searches. All authors commented on and edited the manuscript. FJB, LFPN, and SM edited the final and revised draft.

Declaration of interests

We declare no competing interests.

References

- Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952–53. I. Clinical features. *Trans R Soc Trop Med Hyg* 1955; **49**: 28–32.
- Suhrbier A, Jaffar-Bandjee M-C, Gasque P. Arthritogenic alphaviruses—an overview. *Nat Rev Rheumatol* 2012; **8**: 420–29.
- Joubert JJ, Prozesky OW, Lourens JG, et al. Prevalence of hepatitis virus and some arbovirus infections in Kavango, northern SWA/Namibia. *S Afr Med J* 1985; **67**: 500–02.
- Josseran L, Paquet C, Zehgnoun A, et al. Chikungunya disease outbreak, Reunion Island. *Emerg Infect Dis* 2006; **12**: 1994–95.
- Rampal SM, Meena H. Neurological complications in chikungunya fever. *J Assoc Physicians India* 2007; **55**: 765–69.
- Wielanek AC, Monredon JD, Amrani ME, Roger JC, Serveaux JP. Guillain-Barre syndrome complicating a chikungunya virus infection. *Neurology* 2007; **69**: 2105–07.
- Diallo M, Thonnon J, Traore-Lamizana M, Fontenille D. Vectors of chikungunya virus in Senegal: current data and transmission cycles. *Am J Trop Med Hyg* 1999; **60**: 281–86.
- Tsetsarkin KA, Vanlandingham DL, McGee CE, Higgs S. A single mutation in chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathog* 2007; **3**: e201.
- Weaver SC, Forrester NL. Chikungunya: evolutionary history and recent epidemic spread. *Antiviral Res* 2015; **120**: 32–39.
- Rezza G, Nicoletti L, Angelini R, et al. Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet* 2007; **370**: 1840–46.
- Gould EA, Gallian P, De Lamballerie X, Charrel RN. First cases of autochthonous dengue fever and chikungunya fever in France: from bad dream to reality! *Clin Microbiol Infect* 2010; **16**: 1702–04.
- Delisle E, Rousseau C, Broche B, et al. Chikungunya outbreak in Montpellier, France, September to October 2014. *Euro Surveill* 2015; **20**: 21108.
- Cassadou S, Boucau S, Petit-Sinturel M, Huc P, Leparç-Goffart I, Ledrans M. Emergence of chikungunya fever on the French side of Saint Martin island, October to December 2013. *Euro Surveill* 2014; **19**: 20752.
- Leparç-Goffart I, Nougaière A, Cassadou S, Prat C, de Lamballerie X. Chikungunya in the Americas. *Lancet* 2014; **383**: 514.
- Van Bortel W, Dorleans F, Rosine J, et al. Chikungunya outbreak in the Caribbean region, December 2013 to March 2014, and the significance for Europe. *Euro Surveill* 2014; **19**: 20759.
- Petersen LR, Powers AM. Chikungunya: epidemiology. *F1000Research* 2016; **5**: 82.
- Chen R, Wang E, Tsetsarkin KA, Weaver SC. Chikungunya virus 3′ untranslated region: adaptation to mosquitoes and a population bottleneck as major evolutionary forces. *PLoS Pathog* 2013; **9**: e1003591.
- Van Duijl-Richter MKS, Hoornweg TE, Rodenhuis-Zybert IA, Smit JM. Early events in chikungunya virus infection—from virus cell binding to membrane fusion. *Viruses* 2015; **7**: 3647–74.
- Utt A, Das PK, Varjak M, Lulla V, Lulla A, Merits A. Mutations conferring a noncytotoxic phenotype on chikungunya virus replicons compromise enzymatic properties of nonstructural protein 2. *J Virol* 2015; **89**: 3145–62.
- Karpe YA, Aher PP, Lole KS. NTPase and 5′-RNA triphosphatase activities of chikungunya virus nsP2 protein. *PLoS One* 2011; **6**: e22336.
- Das PK, Merits A, Lulla A. Functional cross-talk between distant domains of chikungunya virus non-structural protein 2 is decisive for its RNA-modulating activity. *J Biol Chem* 2014; **289**: 5635–53.
- Akhrymuk I, Kulemzin S V, Frolova EI. Evasion of the innate immune response: the Old World alphavirus nsP2 protein induces rapid degradation of Rpb1, a catalytic subunit of RNA polymerase II. *J Virol* 2012; **86**: 7180–91.
- Fros JJ, Liu WJ, Prow NA, et al. Chikungunya virus nonstructural protein 2 inhibits type I/II interferon-stimulated JAK-STAT signaling. *J Virol* 2010; **84**: 10877–87.
- Fros JJ, van der Maten E, Vlak JM, Pijlman GP. The C-terminal domain of chikungunya virus nsP2 independently governs viral RNA replication, cytopathicity, and inhibition of interferon signaling. *J Virol* 2013; **87**: 10394–400.
- Fros JJ, Major LD, Scholte FEM, et al. Chikungunya virus non-structural protein 2-mediated host shut-off disables the unfolded protein response. *J Gen Virol* 2015; **96**: 580–89.
- Judith D, Mostowy S, Bourai M, et al. Species-specific impact of the autophagy machinery on chikungunya virus infection. *EMBO Rep* 2013; **14**: 534–44.
- Malet H, Coutard B, Jamal S, et al. The crystal structures of chikungunya and Venezuelan equine encephalitis virus nsP3 macro domains define a conserved adenosine binding pocket. *J Virol* 2009; **83**: 6534–45.
- Panas MD, Ahola T, McInerney GM. The C-terminal repeat domains of nsP3 from the Old World alphaviruses bind directly to G3BP. *J Virol* 2014; **88**: 5888–93.

- 29 Neuvonen M, Kazlauskas A, Martikainen M, Hinkkanen A, Ahola T, Saksela K. SH3 domain-mediated recruitment of host cell amphiphysins by alphavirus nsP3 promotes viral RNA replication. *PLoS Pathog* 2011; **7**: e1002383.
- 30 Bouraï M, Lucas-Hourani M, Gad HH, et al. Mapping of chikungunya virus interactions with host proteins identified nsP2 as a highly connected viral component. *J Virol* 2012; **86**: 3121–34.
- 31 Treffers EE, Tas A, Scholte FEM, et al. Temporal SILAC-based quantitative proteomics identifies host factors involved in chikungunya virus replication. *Proteomics* 2015; **15**: 2267–80.
- 32 Das I, Basantray I, Mamidi P, et al. Heat shock protein 90 positively regulates chikungunya virus replication by stabilizing viral non-structural protein nsP2 during infection. *PLoS One* 2014; **9**: e100531.
- 33 Rathore APS, Haystead T, Das PK, Merits A, Ng M-L, Vasudevan SG. Chikungunya virus nsP3 & nsP4 interacts with HSP-90 to promote virus replication: HSP-90 inhibitors reduce CHIKV infection and inflammation in vivo. *Antiviral Res* 2014; **103**: 7–16.
- 34 Dickson AM, Anderson JR, Barnhart MD, et al. Dephosphorylation of HuR protein during alphavirus infection is associated with HuR relocalization to the cytoplasm. *J Biol Chem* 2012; **287**: 36229–38.
- 35 Panas MD, Varjak M, Lulla A, et al. Sequestration of G3BP coupled with efficient translation inhibits stress granules in Semliki Forest virus infection. *Mol Biol Cell* 2012; **23**: 4701–12.
- 36 Scholte FEM, Tas A, Albulescu IC, et al. Stress granule components G3BP1 and G3BP2 play a proviral role early in chikungunya virus replication. *J Virol* 2015; **89**: 4457–69.
- 37 Rupp JC, Sokolowski KJ, Gebhart NN, Hardy RW. Alphavirus RNA synthesis and nonstructural protein functions. *J Gen Virol* 2015; **96**: 2483–500.
- 38 Kim DY, Firth AE, Atasheva S, Frolova EI, Frolov I. Conservation of a packaging signal and the viral genome RNA packaging mechanism in alphavirus evolution. *J Virol* 2011; **85**: 8022–36.
- 39 Scholte FEM, Tas A, Martina BEE, et al. Characterization of synthetic chikungunya viruses based on the consensus sequence of recent E1-226V isolates. *PLoS One* 2013; **8**: e71047.
- 40 Thomas S, Rai J, John L, Schaefer S, Pützer BM, Herchenröder O. Chikungunya virus capsid protein contains nuclear import and export signals. *Virol J* 2013; **10**: 269.
- 41 Ooi YS, Dubé M, Kielian M. BST2/tetherin inhibition of alphavirus exit. *Viruses* 2015; **7**: 2147–67.
- 42 Jones PH, Maric M, Madison MN, Maury W, Roller RJ, Okeoma CM. BST-2/tetherin-mediated restriction of chikungunya (CHIKV) VLP budding is counteracted by CHIKV non-structural protein 1 (nsP1). *Virology* 2013; **438**: 37–49.
- 43 Lim PJ, Chu JHH. A polarized cell model for chikungunya virus infection: entry and egress of virus occurs at the apical domain of polarized cells. *PLoS Negl Trop Dis* 2014; **8**: e2661.
- 44 Filipe AF, Pinto MR. Arbovirus studies in Luanda, Angola. 2. Virological and serological studies during an outbreak of dengue-like disease caused by the Chikungunya virus. *Bull World Health Organ* 1973; **49**: 37–40.
- 45 Eisenhut M, Schwarz TF, Hegenscheid B. Seroprevalence of dengue, chikungunya and Sindbis virus infections in German aid workers. *Infection* 1999; **27**: 82–85.
- 46 Rodhain F, Carteron B, Laroche R, Hannoun C. Human arbovirus infections in Burundi: results of a seroepidemiologic survey, 1980–1982. *Bull Soc Pathol Exot Filiales* 1987; **80**: 155–61 (in French).
- 47 Kuniholm MH, Wolfe ND, Huang CY-H, et al. Seroprevalence and distribution of Flaviviridae, Togaviridae, and Bunyaviridae arboviral infections in rural Cameroonian adults. *Am J Trop Med Hyg* 2006; **74**: 1078–83.
- 48 Peyrefitte CN, Rousset D, Pastorino BAM, et al. Chikungunya virus, Cameroon, 2006. *Emerg Infect Dis* 2007; **13**: 768–71.
- 49 Guilherme JM, Gonella-Legall C, Legall F, Nakoume E, Vincent J. Seroprevalence of five arboviruses in Zebu cattle in the Central African Republic. *Trans R Soc Trop Med Hyg* 1990; **90**: 31–3.
- 50 Pastorino B, Muyembe-Tamfum JJ, Bessaud M, et al. Epidemic resurgence of Chikungunya virus in democratic Republic of the Congo: identification of a new central African strain. *J Med Virol* 2004; **74**: 277–82.
- 51 Peyrefitte CN, Bessaud M, Pastorino BAM, et al. Circulation of chikungunya virus in Gabon, 2006–2007. *J Med Virol* 2008; **80**: 430–3.
- 52 Jentes ES, Robinson J, Johnson BW, et al. Acute arboviral infections in Guinea, West Africa, 2006. *Am J Trop Med Hyg* 2010; **83**: 388–94.
- 53 Serگون K, Njuguna C, Kalani R, et al. Seroprevalence of chikungunya virus (CHIKV) infection on Lamu Island, Kenya, October 2004. *Am J Trop Med Hyg* 2008; **78**: 333–37.
- 54 Kaschula VR, Van Dellen AF, de Vos V. Some infectious diseases of wild vervet monkeys (*Cercopithecus aethiops pygerythrus*) in South Africa. *J S Afr Vet Assoc* 1978; **49**: 223–37.
- 55 Pistone T, Ezzedine K, Schuffenecker I, Receveur M-C, Malvy D. An imported case of chikungunya fever from Madagascar: use of the sentinel traveller for detecting emerging arboviral infections in tropical and European countries. *Travel Med Infect Dis* 2009; **7**: 52–54.
- 56 Van den Bosch C, Lloyd G. Chikungunya fever as a risk factor for endemic Burkitt's lymphoma in Malawi. *Trans R Soc Trop Med Hyg* 2000; **94**: 704–05.
- 57 Moore DL, Causey OR, Carey DE, et al. Arthropod-borne viral infections of man in Nigeria, 1964–1970. *Ann Trop Med Parasitol* 1975; **69**: 49–64.
- 58 Weinbren MP, Haddow AJ, Williams MC. The occurrence of Chikungunya virus in Uganda. I. Isolation from mosquitoes. *Trans R Soc Trop Med Hyg* 1958; **52**: 253–57.
- 59 Pistone T, Ezzedine K, Boisvert M, et al. Cluster of chikungunya virus infection in travelers returning from Senegal, 2006. *J Travel Med* 2006; **13**: 286–88.
- 60 Woodruff AW, Bowen ET, Platt GS. Viral infections in travellers from tropical Africa. *BMJ* 1978; **1**: 956–58.
- 61 Gould LH, Osman MS, Farnon EC, et al. An outbreak of yellow fever with concurrent chikungunya virus transmission in South Kordofan, Sudan, 2005. *Trans R Soc Trop Med Hyg* 2008; **102**: 1247–54.
- 62 Lahariya C, Pradhan SK. Emergence of chikungunya virus in Indian subcontinent after 32 years: a review. *J Vector Borne Dis* 2006; **43**: 151–60.
- 63 Rao TR. Immunological surveys of arbovirus infections in South-East Asia, with special reference to dengue, chikungunya, and Kyasanur Forest disease. *Bull World Health Organ* 1971; **44**: 585–91.
- 64 Mackenzie JS, Chua KB, Daniels PW, et al. Emerging viral diseases of Southeast Asia and the Western Pacific. *Emerg Infect Dis* 2001; **7**: 497–504.
- 65 Sarkar JK, Pavri KM, Chatterjee SN, Chakravarty SK, Anderson CR. Virological and serological studies of cases of haemorrhagic fever in Calcutta. Material collected by the Calcutta School of Tropical Medicine. *Indian J Med Res* 1964; **52**: 684–91.
- 66 Wangchuk S, Chinnawirotpisan P, Dorji T, et al. Chikungunya fever outbreak, Bhutan, 2012. *Emerg Infect Dis* 2013; **19**: 1681–84.
- 67 Hussain R, Alomar I, Memish ZA. Chikungunya virus: emergence of an arthritic arbovirus in Jeddah, Saudi Arabia. *East Mediterr Health J* 2013; **19**: 506–08.
- 68 Rezza G, El-Sawaf G, Faggioni G, et al. Co-circulation of dengue and chikungunya viruses, Al Hudaydah, Yemen, 2012. *Emerg Infect Dis* 2014; **20**: 1351–54.
- 69 Powers AM, Brault AC, Tesh RB, Weaver SC. Re-emergence of chikungunya and o'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *J Gen Virol* 2000; **81**: 471–79.
- 70 Arias-Goeta C, Mousson L, Rougeon F, Failloux A-B. Dissemination and transmission of the E1–226V variant of chikungunya virus in *Aedes albopictus* are controlled at the midgut barrier level. *PLoS One* 2013; **8**: e57548.
- 71 Laras K, Sukri NC, Larasati RP, et al. Tracking the re-emergence of epidemic chikungunya virus in Indonesia. *Trans R Soc Trop Med Hyg* 2005; **99**: 128–41.
- 72 Serگون K, Yahaya AA, Brown J, et al. Seroprevalence of chikungunya virus infection on Grande Comore Island, union of the Comoros, 2005. *Am J Trop Med Hyg* 2007; **76**: 1189–93.
- 73 Pialoux G, Gaüzère B-A, Jauréguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis* 2007; **7**: 319–27.
- 74 Renault P, Solet J-L, Sissoko D, et al. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005–2006. *Am J Trop Med Hyg* 2007; **77**: 727–31.

- 75 D'Ortenzio E, Grandadam M, Balleydier E, et al. Sporadic cases of chikungunya, Réunion Island, August 2009. *Euro Surveill* 2009; **14**: 19324.
- 76 D'Ortenzio E, Grandadam M, Balleydier E, et al. A226V strains of chikungunya virus, Réunion Island, 2010. *Emerg Infect Dis* 2011; **17**: 309–11.
- 77 Arankalle VA, Shrivastava S, Cherian S, et al. Genetic divergence of chikungunya viruses in India (1963–2006) with special reference to the 2005–2006 explosive epidemic. *J Gen Virol* 2007; **88**: 1967–76.
- 78 Mourya DT, Thakare JR, Gokhale MD, et al. Isolation of chikungunya virus from *Aedes aegypti* mosquitoes collected in the town of Yawat, Pune District, Maharashtra State, India. *Acta Virol* 2001; **45**: 305–09.
- 79 Vazeille M, Moutailler S, Coudrier D, et al. Two chikungunya isolates from the outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito, *Aedes albopictus*. *PLoS One* 2007; **2**: e1168.
- 80 Tssetsarkin KA, Weaver SC. Sequential adaptive mutations enhance efficient vector switching by chikungunya virus and its epidemic emergence. *PLoS Pathog* 2011; **7**: e1002412.
- 81 Tssetsarkin KA, Chen R, Leal G, et al. Chikungunya virus emergence is constrained in Asia by lineage-specific adaptive landscapes. *Proc Natl Acad Sci USA* 2011; **108**: 7872–77.
- 82 Sy AK, Saito-Obata M, Medado IA, et al. Molecular characterization of chikungunya virus, Philippines, 2011–2013. *Emerg Infect Dis* 2016; **22**: 887–90.
- 83 Lanciotti RS, Valadere AM. Transcontinental movement of Asian genotype chikungunya virus. *Emerg Infect Dis* 2014; **20**: 1400–02.
- 84 Nunes MRT, Faria NR, de Vasconcelos JM, et al. Emergence and potential for spread of chikungunya virus in Brazil. *BMC Med* 2015; **13**: 102.
- 85 Merkle SH, van Rij RP. Beyond RNAi: antiviral defense strategies in *Drosophila* and mosquito. *J Insect Physiol* 2013; **59**: 159–70.
- 86 McFarlane M, Arias-Goeta C, Martin E, et al. Characterization of *Aedes aegypti* innate-immune pathways that limit Chikungunya virus replication. *PLoS Negl Trop Dis* 2014; **8**: e2994.
- 87 Donald CL, Kohl A, Schnettler E. New insights into control of arbovirus replication and spread by insect RNA interference pathways. *Insects* 2012; **3**: 511–31.
- 88 Morazzani EM, Wiley MR, Murreddu MG, Adelman ZN, Myles KM. Production of virus-derived ping-pong-dependent piRNA-like small RNAs in the mosquito soma. *PLoS Pathog* 2012; **8**: e1002470.
- 89 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281–97.
- 90 Shrinet J, Jain S, Jain J, Bhatnagar RK, Sunil S. Next generation sequencing reveals regulation of distinct *Aedes* microRNAs during chikungunya virus development. *PLoS Negl Trop Dis* 2014; **8**: e2616.
- 91 Maharaj PD, Widen SG, Huang J, Wood TG, Thangamani S. Discovery of mosquito saliva microRNAs during CHIKV infection. *PLoS Negl Trop Dis* 2015; **9**: e0003386.
- 92 Miner JJ, Aw Yeang HX, Fox JM, et al. Brief report: chikungunya viral arthritis in the United States: a mimic of seronegative rheumatoid arthritis. *Arthritis Rheumatol* 2015; **67**: 1214–20.
- 93 Mahendradas P, Avadhani K, Shetty R. Chikungunya and the eye: a review. *J Ophthalmic Inflamm Infect* 2013; **3**: 35.
- 94 Mohan A, Kiran DHN, Manohar IC, Kumar DP. Epidemiology, clinical manifestations, and diagnosis of Chikungunya fever: lessons learned from the re-emerging epidemic. *Indian J Dermatol* 2010; **55**: 54–63.
- 95 Simon F, Javelle E, Oliver M, Leparco-Goffart I, Marimoutou C. Chikungunya virus infection. *Curr Infect Dis Rep* 2011; **13**: 218–28.
- 96 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**: 1401–07.
- 97 Brighton SW, Prozesky OW, de la Harpe AL. Chikungunya virus infection. A retrospective study of 107 cases. *S Afr Med J* 1983; **63**: 313–15.
- 98 Hochedez P, Jaureguiberry S, Debruyne M, et al. Chikungunya infection in travelers. *Emerg Infect Dis* 2006; **12**: 1565–67.
- 99 Marimoutou C, Vivier E, Oliver M, Boutin J-P, Simon F. Morbidity and impaired quality of life 30 months after chikungunya infection: comparative cohort of infected and uninfected French military policemen in Reunion Island. *Medicine* 2012; **91**: 212–19.
- 100 Schilte C, Staikowsky F, Staikowsky F, et al. Chikungunya virus-associated long-term arthralgia: a 36-month prospective longitudinal study. *PLoS Negl Trop Dis* 2013; **7**: e2137.
- 101 Rajapakse S, Rodrigo C, Rajapakse A. Atypical manifestations of chikungunya infection. *Trans R Soc Trop Med Hyg* 2010; **104**: 89–96.
- 102 Singh SS, Manimunda SP, Sugunan AP, Sahina, Vijayachari P. Four cases of acute flaccid paralysis associated with chikungunya virus infection. *Epidemiol Infect* 2008; **136**: 1277–80.
- 103 Lemant J, Boisson V, Winer A, et al. Serious acute chikungunya virus infection requiring intensive care during the Reunion Island outbreak in 2005–2006. *Crit Care Med* 2008; **36**: 2536–41.
- 104 Ramful D, Carbonnier M, Pasquet M, et al. Mother-to-child transmission of chikungunya virus infection. *Pediatr Infect Dis J* 2007; **26**: 811–15.
- 105 Gérardin P, Sampéris S, Ramful D, et al. Neurocognitive outcome of children exposed to perinatal mother-to-child chikungunya virus infection: the CHIMERE cohort study on Reunion Island. *PLoS Negl Trop Dis* 2014; **8**: e2996.
- 106 Brighton SW, Simson IW. A destructive arthropathy following chikungunya virus arthritis—a possible association. *Clin Rheumatol* 1984; **3**: 253–58.
- 107 Malvy D, Ezzedine K, Mamani-Matsuda M, et al. Destructive arthritis in a patient with chikungunya virus infection with persistent specific IgM antibodies. *BMC Infect Dis* 2009; **9**: 200.
- 108 Manimunda SP, Vijayachari P, Uppoor R, et al. Clinical progression of chikungunya fever during acute and chronic arthritic stages and the changes in joint morphology as revealed by imaging. *Trans R Soc Trop Med Hyg* 2010; **104**: 392–99.
- 109 Chirathaworn C, Poovorawan Y, Lertmaharit S, Wuttirattanakowit N. Cytokine levels in patients with chikungunya virus infection. *Asian Pac J Trop Med* 2013; **6**: 631–34.
- 110 Kelvin AA, Banner D, Silvi G, et al. Inflammatory cytokine expression is associated with chikungunya virus resolution and symptom severity. *PLoS Negl Trop Dis* 2011; **5**: e1279.
- 111 Reddy V, Mani RS, Desai A, Ravi V. Correlation of plasma viral loads and presence of Chikungunya IgM antibodies with cytokine/chemokine levels during acute chikungunya virus infection. *J Med Virol* 2014; **86**: 1393–401.
- 112 Teng T-S, Kam Y-W, Lee B, et al. A systematic meta-analysis of immune signatures in patients with acute chikungunya virus infection. *J Infect Dis* 2015; **211**: 1925–35.
- 113 Teo T-H, Lum F-M, Claser C, et al. A pathogenic role for CD4+ T cells during chikungunya virus infection in mice. *J Immunol* 2013; **190**: 259–69.
- 114 Seymour RL, Adams AP, Leal G, Alcorn MDH, 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.
- 115 Kam Y-W, Lee WWL, Simarmata D, et al. Longitudinal analysis of the human antibody response to chikungunya virus infection: implications for serodiagnosis and vaccine development. *J Virol* 2012; **86**: 13005–15.
- 116 Smith SA, Silva LA, Fox JM, et al. Isolation and characterization of broad and ultrapotent human monoclonal antibodies with therapeutic activity against chikungunya virus. *Cell Host Microbe* 2015; **18**: 86–95.
- 117 Noret M, Herrero L, Rulli N, et al. Interleukin 6, RANKL, and osteoprotegerin expression by chikungunya virus-infected human osteoblasts. *J Infect Dis* 2012; **206**: 455–57: 457–59.
- 118 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.
- 119 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**: 782–85.
- 120 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.
- 121 Chen C-I, 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.

- 122 Messaoudi I, Vomazke 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.
- 123 Her Z, Teng T-S, Tan JLL, et al. Loss of TLR3 aggravates CHIKV replication and pathology due to an altered virus-specific neutralizing antibody response. *EMBO Mol Med* 2015; **7**: 24–41.
- 124 Chen W, Foo S-S, Taylor A, et al. Bindarit, an inhibitor of monocyte chemotactic protein synthesis, protects against bone loss induced by chikungunya virus infection. *J Virol* 2015; **89**: 581–93.
- 125 Poo YS, Nakaya H, Gardner J, et al. CCR2 deficiency promotes exacerbated chronic erosive neutrophil-dominated chikungunya virus arthritis. *J Virol* 2014; **88**: 6862–72.
- 126 Herrero LJ, Sheng K-C, Jian P, et al. Macrophage migration inhibitory factor receptor CD74 mediates alphavirus-induced arthritis and myositis in murine models of alphavirus infection. *Arthritis Rheum* 2013; **65**: 2724–36.
- 127 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.
- 128 White LK, Sali T, Alvarado D, et al. Chikungunya virus induces IPS-1-dependent innate immune activation and protein kinase R-independent translational shutoff. *J Virol* 2011; **85**: 606–20.
- 129 Nakaya HI, Gardner J, Poo Y-S, Major L, Pulendran B, Suhrbier A. Gene profiling of chikungunya virus arthritis in a mouse model reveals significant overlap with rheumatoid arthritis. *Arthritis Rheum* 2012; **64**: 3553–63.
- 130 Hawman DW, Stoermer K, 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.
- 131 De Lamballerie X, Ninove L, Charrel RN. Antiviral treatment of chikungunya virus infection. *Infect Disord Drug Targets* 2009; **9**: 101–04.
- 132 Briolant S, Garin D, Scaramozzino N, Jouan A, Crance JM. In vitro inhibition of chikungunya and Semliki Forest viruses replication by antiviral compounds: synergistic effect of interferon-alpha and ribavirin combination. *Antiviral Res* 2004; **61**: 111–17.
- 133 Khan M, Dhanwani R, Patro IK, Rao PVL, Parida MM. Cellular IMPDH enzyme activity is a potential target for the inhibition of chikungunya virus replication and virus induced apoptosis in cultured mammalian cells. *Antiviral Res* 2011; **89**: 1–8.
- 134 Kaur P, Chu JHH. Chikungunya virus: an update on antiviral development and challenges. *Drug Discov Today* 2013; **18**: 969–83.
- 135 Delang L, Segura Guerrero N, Tas A, et al. Mutations in the chikungunya virus non-structural proteins cause resistance to favipiravir (T-705), a broad-spectrum antiviral. *J Antimicrob Chemother* 2014; **69**: 2770–84.
- 136 Bassetto M, De Burghgraeve T, Delang L, et al. Computer-aided identification, design and synthesis of a novel series of compounds with selective antiviral activity against chikungunya virus. *Antiviral Res* 2013; **98**: 12–18.
- 137 Allard P-M, Leyssen P, Martin M-T, et al. Antiviral chlorinated daphnane diterpenoid orthoesters from the bark and wood of *Trigonostemon cherrieri*. *Phytochemistry* 2012; **84**: 160–68.
- 138 Bourjot M, Delang L, Nguyen VH, et al. Prostratin and 12-O-tetradecanoylphorbol 13-acetate are potent and selective inhibitors of chikungunya virus replication. *J Nat Prod* 2012; **75**: 2183–87.
- 139 Pohjala L, Alakurtti S, Ahola T, Yli-Kauhaluoma J, Tammela P. Betulin-derived compounds as inhibitors of alphavirus replication. *J Nat Prod* 2009; **72**: 1917–26.
- 140 Cruz DJM, Bonotto RM, Gomes RGB, et al. Identification of novel compounds inhibiting chikungunya virus-induced cell death by high throughput screening of a kinase inhibitor library. *PLoS Negl Trop Dis* 2013; **7**: e2471.
- 141 Harrison VR, Eckels KH, Bartelloni PJ, Hampton C. Production and evaluation of a formalin-killed chikungunya vaccine. *J Immunol* 1971; **107**: 643–47.
- 142 DeMeio JL, DeSanctis AN, Thomas WJ. Persistence in humans of antibody after immunization with four alphavirus vaccines. *Asian J Infect Dis* 1979; **3**: 119–24.
- 143 Levitt NH, Ramsburg HH, Hasty SE, Repik PM, Cole FE, Lupton HW. Development of an attenuated strain of chikungunya virus for use in vaccine production. *Vaccine* 1986; **4**: 157–62.
- 144 Hoke CH, Pace-Templeton J, Pittman P, et al. US Military contributions to the global response to pandemic chikungunya. *Vaccine* 2012; **30**: 6713–20.
- 145 Chu H, Das SC, Fuchs JF, et al. Deciphering the protective role of adaptive immunity to CHIKV/IRES a novel candidate vaccine against chikungunya in the A129 mouse model. *Vaccine* 2013; **31**: 3353–60.
- 146 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**: e1002142.
- 147 Partidos CD, Paykel J, Weger J, et al. Cross-protective immunity against o'nyong-nyong virus afforded by a novel recombinant chikungunya vaccine. *Vaccine* 2012; **30**: 4638–43.
- 148 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**: 1891–99.
- 149 Hallengård 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.
- 150 Akahata W, Yang Z-Y, Andersen H, et al. A virus-like particle vaccine for epidemic chikungunya virus protects nonhuman primates against infection. *Nat Med* 2010; **16**: 334–38.
- 151 Metz SW, Pijlman GP. Arbovirus vaccines; opportunities for the baculovirus-insect cell expression system. *J Invertebr Pathol* 2011; **107** (suppl): 16–30.
- 152 Metz SW, Martina BE, van den Doel P, et al. Chikungunya virus-like particles are more immunogenic in a lethal AG129 mouse model compared to glycoprotein E1 or E2 subunits. *Vaccine* 2013; **31**: 6092–96.
- 153 Akahata W, Nabel GJ. A specific domain of the chikungunya virus E2 protein regulates particle formation in human cells: implications for alphavirus vaccine design. *J Virol* 2012; **86**: 8879–83.
- 154 Brandler S, Ruffié C, Combredet C, et al. A recombinant measles vaccine expressing chikungunya virus-like particles is strongly immunogenic and protects mice from lethal challenge with chikungunya virus. *Vaccine* 2013; **31**: 3718–25.
- 155 Chang L-J, Dowd KA, Mendoza FH, et al. Safety and tolerability of chikungunya virus-like particle vaccine in healthy adults: a phase 1 dose-escalation trial. *Lancet* 2014; **384**: 2046–52.
- 156 Ramsauer K, Schwameis M, Firbas C, et al. Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial. *Lancet Infect Dis* 2015; **15**: 519–27.
- 157 Wang D, Suhrbier A, Penn-Nicholson A, et al. A complex adenovirus vaccine against chikungunya virus provides complete protection against viraemia and arthritis. *Vaccine* 2011; **29**: 2803–09.
- 158 Chattopadhyay A, Wang E, Seymour R, Weaver SC, Rose JK. A chimeric vesiculo/alphavirus is an effective alphavirus vaccine. *J Virol* 2013; **87**: 395–402.
- 159 García-Arriaza J, Cepeda V, Hallengård D, et al. A novel poxvirus-based vaccine, MVA-CHIKV, is highly immunogenic and protects mice against chikungunya infection. *J Virol* 2014; **88**: 3527–47.
- 160 Muthumani K, Lankaraman KM, Laddy DJ, et al. Immunogenicity of novel consensus-based DNA vaccines against chikungunya virus. *Vaccine* 2008; **26**: 5128–34.
- 161 Mallilankaraman K, Shedlock DJ, Bao H, et al. A DNA vaccine against chikungunya virus is protective in mice and induces neutralizing antibodies in mice and nonhuman primates. *PLoS Negl Trop Dis* 2011; **5**: e928.
- 162 Bao H, Ramanathan AA, Kawalakar O, et al. Nonstructural protein 2 (nsP2) of chikungunya virus (CHIKV) enhances protective immunity mediated by a CHIKV envelope protein expressing DNA vaccine. *Viral Immunol* 2013; **26**: 75–83.
- 163 Khan M, Dhanwani R, Rao PVL, Parida M. Subunit vaccine formulations based on recombinant envelope proteins of chikungunya virus elicit balanced Th1/Th2 response and virus-neutralizing antibodies in mice. *Virus Res* 2012; **167**: 236–46.
- 164 Kumar M, Sudeep AB, Arankalle VA. Evaluation of recombinant E2 protein-based and whole-virus inactivated candidate vaccines against chikungunya virus. *Vaccine* 2012; **30**: 6142–49.
- 165 Couderc T, Khandoudi N, Grandadam M, et al. Prophylaxis and therapy for chikungunya virus infection. *J Infect Dis* 2009; **200**: 516–23.

- 166 Fric J, Bertin-Maghit S, Wang C-I, Nardin A, Warter L. Use of human monoclonal antibodies to treat chikungunya virus infection. *J Infect Dis* 2013; **207**: 319–22.
- 167 Selvarajah S, Sexton NR, Kahle KM, et al. A neutralizing monoclonal antibody targeting the acid-sensitive region in chikungunya virus E2 protects from disease. *PLoS Negl Trop Dis* 2013; **7**: e2423.
- 168 Fox JM, Long F, Edeling MA, et al. Broadly neutralizing alphavirus antibodies bind an epitope on E2 and inhibit entry and egress. *Cell* 2015; **163**: 1095–107.

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