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Chikungunya Virus

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KEYWORDS

• Chikungunya virus • Alphavirus • Arbovirus • Togaviridae

KEY POINTS

- Chikungunya is an arboviral infection that causes debilitating arthritis and arthralgia.
- Chikungunya virus has caused explosive epidemics in the past decade, and has spread rapidly from Africa to Asia to the Americas.
- Improved diagnostic testing and surveillance for chikungunya infection is needed to detect and respond to future outbreaks.
- Further investigation into the pathogenesis of chikungunya infection is needed to understand its long-term sequelae, and to develop effective therapies.

MICROBIOLOGY

Chikungunya virus (CHIKV) belongs to the Semliki Forest antigenic group of the genus *Alphaviridae*, which includes other arthritogenic alphaviruses, such as o'nyong-nyong, Ross River, Barmah Forest, and Mayaro viruses.[1,2] Its genome is closely related to that of o'nyong-nyong virus, and consists of a single 11.8-kbp strand of positive sense RNA, which encodes a 2472 amino acid nonstructural and a 1244 amino acid structural polyprotein.[3] The polyproteins give rise to the four nonstructural proteins (nsP1-4) that make up the viral replication machine, and five structural proteins. Each spherical viral particle is approximately 70 nm in diameter and is comprised of a strand of genomic RNA, encapsidated by capsid (C) proteins, surrounded by a host cell–derived lipid bilayer spiked with heterodimers of envelope proteins E1 and E2.[4] The other two structural proteins, 6K and E3, are leader peptides for E1 and E2, respectively, and are not observed in abundance in the mature virion.[4]

Disclosure Statement: The authors have no relevant disclosures.
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Clin Lab Med 37 (2017) 371–382
http://dx.doi.org/10.1016/j.cll.2017.01.008 labmed.theclinics.com

The envelope proteins, E2 and E1, play important roles in the binding of the virus to the host cell membrane and its subsequent cellular invasion, respectively. Anti-CHIKV antibodies directed against the envelope protein that neutralize the virus in vitro also protect neonatal mice from lethal CHIKV infection in vivo, suggesting that these proteins may be important antigenic lethal targets for development of naturally acquired, or vaccine-elicited protection.[5–7]

EPIDEMIOLOGY

The earliest report of chikungunya fever described an outbreak of a dengue-like illness that occurred in 1952 to 1953, on the Makonde Plateau in the Southern Province of Tanganyika (present day Tanzania).[8] Residents of all ages experienced a febrile illness with rash and arthralgia. However, certain aspects of this outbreak distinguished it from previous reports of dengue fever outbreaks. Most striking was the severity of the arthralgia that "would prevent the sufferer from changing position without help."[8] The local population began to call the disease chikungunya, which is a Makonde (Bantu) term that means "that which bends up," referring to the contorted positions of those who were affected by the sudden and severe onset of arthralgia. Additionally, many individuals affected with the disease continued to experience intermittent joint pains that persisted for months after the acute illness. The attack rate also seemed to be unusually high, often affecting entire households. Between 1952 and 1953, an estimated 60% to 80% of the population in this region developed symptoms of fever, rash, and arthralgia.[8] Attempts to isolate the pathologic agent from symptomatic individuals during the outbreak also diverged from previous experience with dengue virus (DENV): Inoculation of infant mice with serum samples from symptomatic individuals resulted in death of the animals. In contrast, DENV infection is difficult to establish in mice.[9] These data suggested that the cause of the syndrome termed chikungunya indeed was distinct from the cause of dengue fever.

In Africa, CHIKV is transmitted by arboreal *Aedes* mosquitoes (*A furcifer-taylori*, *A africanus*, *A luteocephalus*, and *A neoafricanus*) in an enzootic cycle with nonhuman primates as the principle reservoir (**Fig. 1**).[10–12] Between the 1960s and 1990s, incidental human infection led to numerous, small-scale CHIKV outbreaks in countries throughout Central and Southern Africa, and Senegal, Guinea, and Nigeria in Western Africa (reviewed in Ref.[13]). The outbreaks occurred after periods of large rainfall and associated surges in the arboreal *Aedes* mosquito density. In contrast, CHIKV outbreaks in Southeast Asia occurred in larger cities where *Aedes aegypti* mosquitoes were implicated as the primary transmission vector. *A aegypti* mosquitoes require very small amounts of water to lay eggs, and thrive in human urban environments, particularly in areas where residents store water in open containers or cisterns.

In 2004, a large-scale CHIKV epidemic erupted, sweeping down the coast of Kenya into islands on the Indian Ocean (Comoros, Mayotte, Seychelles, Réunion, Madagascar, Sri Lanka, and the Maldives), India, Southeast Asia (Malaysia, Singapore, Thailand), and China.[14] Although CHIKV infection in travelers returning to Europe had been reported previously, autochthonous transmission of CHIKV was observed for the first time in Italy in 2007,[15] and in France in 2009.[16] An important factor that facilitated the rapid expansion of CHIKV infection was a novel single amino acid substitution of alanine for valine at position 226 (A226V) in the E1 envelope protein that enhanced the ability of the *Aedes albopictus* mosquito to transmit CHIKV to humans.[17] *A albopictus* is an anthropophilic, peridomestic species of mosquito that

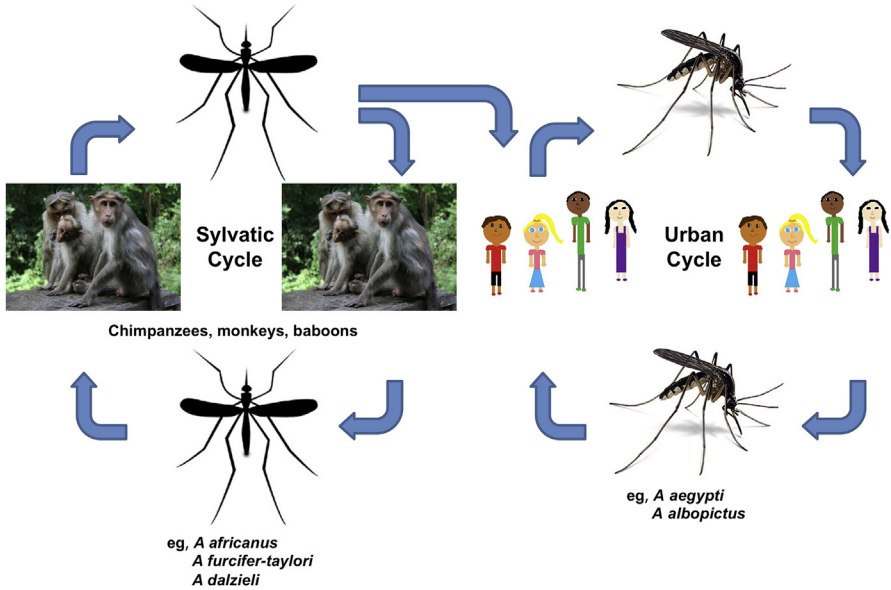

Fig. 1. Transmission cycle of CHIKV in Africa. Nonhuman primates, and possibly other wild animals, serve as reservoirs of the virus. Infected arboreal *Aedes* mosquitoes bite and infect humans. Infected humans, in turn, infect peridomestic *Aedes aegypti*, perpetuating the urban cycle of CHIKV transmission. (*From* Thiboutot MM, Kannan S, Kawalekar OU, et al. Chikungunya: a potentially emerging epidemic? PLoS Negl Trop Dis 2010;4(4):e623.)

has an even greater geographic range than that of its relative, *A aegypti*, and has been implicated as having a major role in the spread of CHIKV epidemics across Asia and to the Americas.

In December 2013, the first cases of locally transmitted CHIKV in the Americas were confirmed in St. Martin,[18] followed rapidly by cases identified throughout the Caribbean and Latin America. By January 2015, CHIKV infection had been identified in 42 countries or territories in the Caribbean, Central America, South America, and North America (local transmission in Florida) with more than a million suspected cases reported, and more than 25,000 laboratory-confirmed.[19]

Although epidemics of febrile arthralgia have been reported in the Americas since the 1700s, these outbreaks previously had been attributed to dengue fever. For example, an epidemic "break-bone fever," which referred to modern-day dengue fever, erupted on the islands of St. Thomas and Santa Cruz in the West Indies from 1827 to 1828. Stedman, who reported this "anomalous disease" called "dandy fever" by local residents, noted that the illness "attacked almost every individual in the town," had "extremely low mortality," and was associated with "pains in the joints for weeks after recovery from the acute stage," which were key differences between the 1827 and 1828 West Indies epidemic and previous descriptions of a "break-bone fever" (referring to modern-day dengue fever). He concluded that "the diseases, though somewhat alike in a few symptoms, are essentially different."[20] Thus, although the 2013 epidemic was the first CHIKV outbreak in the Americas to be confirmed using modern-day virologic methods, historical reports raise questions to whether this truly was the first introduction of CHIKV infection to the Americas.[21]

PATHOGENESIS

CHIKV is known to infect a variety of cell lines in vitro, including Vero cells (from green monkey kidney)[22] and BHK21 baby hamster kidney cells,[23] and various insect cell lines.[24,25] Human cellular tropism was more recently described.[26] Fibroblasts in the dermis, joint capsule, and muscle seem to be the major targets of CHIKV infection in humans.[27] Human epithelial and endothelial cells[26] and muscle progenitors (satellite cells)[28] also have been observed to be infected by CHIKV. Lymphocytes and monocytes seem resistant, yet macrophages seem susceptible to CHIKV infection.[26]

Investigations of disease pathogenesis during human CHIKV infection have been limited, in part, by the lack of relevant and/or accessible models of CHIKV disease. Development of nonhuman primate models of CHIKV infection has been helpful for use in evaluating potential CHIKV vaccines. Rhesus macaques immunized with an investigational CHIKV virus-like particle vaccine were protected against developing viremia after intravenous challenge with 10^{10} PFU of CHIKV, whereas control monkeys that received the mock vaccine developed high levels of viremia after challenge.[29] In a separate study, cynomolgus macaques challenged with a much lower CHIKV inoculum, by either intravenous or intradermal injection, developed viremia, fever, and rash. Viral RNA remained detectable in synovial and muscle tissue for up to 1.5 months after infection, and in lymphoid tissue for up to 3 months.[30] Thus, this model may be useful for studying long-term sequelae of CHIKV infection, such as the prolonged arthralgia experienced by many CHIKV individuals.

Because nonhuman primate models of CHIKV infection are not readily accessible to many investigators, some investigators have developed mouse models to study CHIKV disease. Viral challenge of neonatal wild-type mice results in fatal infection. However, this susceptibility to CHIKV infection wanes quickly and adult wild-type mice are resistant to CHIKV infection. Type I interferon receptor knock-out mice (IFN-$\alpha/\beta R^{-/-}$) have an impaired type I IFN pathway and, in contrast with adult wild-type mice, develop viremia after viral challenge.[27] Thus, it seems that activation of the type I IFN pathway plays an important role in controlling CHIKV infection.

Mice also have been used to develop models of CHIKV-related arthritis.[31,32] One group of investigators demonstrated that mice injected with CHIKV in the footpad developed leg swelling and weight loss, and had histologic evidence of necrotizing myositis, arthritis, tenosynovitis, and vasculitis.[32] A separate group of investigators also elicited foot swelling in mice injected with CHIKV in the ventral footpad. These mice developed viremia, and histologic examination revealed large mononuclear cell infiltrates in and around synovial membranes, and in muscle tissue. Furthermore, treatment with IFN-α before CHIKV inoculation reduced viremia and prevented manifestations of arthritis,[31] again highlighting the importance of type I IFNs in CHIKV virus control.

CLINICAL MANIFESTATIONS

In contrast to DENV, which can cause asymptomatic infection, most individuals with CHIKV infection are symptomatic.[8] However, chikungunya fever shares many similarities with dengue fever. Sudden onset of high fever is typically the initial symptom reported and can appear within 2 days of infection. A rash sometimes is observed and is typically maculopapular, although bullous rashes have been noted in some infants with CHIKV infection.[33] Both viral infections also are known to cause arthralgia and arthritis. However, the polyarthralgia caused by CHIKV frequently is characterized as debilitating and has been reported to continue well beyond the resolution of fever. For example, a third of travelers to the Caribbean who acquired

CHIKV infection in 2014 while abroad reported persistent joint or muscle pain, or joint swelling at greater than or equal to 9 months after their acute infection.[34] The knees are the most commonly involved joints; however, other large or small joints may be affected. Of note, symmetric involvement of joints is frequently reported.[35] Additional symptoms reported include fatigue, nausea, vomiting, and conjunctivitis. Conspicuously absent among those with CHIKV infection are reports of retro-orbital headache, which are characteristic of dengue fever.[8] Most symptoms resolve within 7 to 10 days; however, many infected individuals have reported protracted arthralgia that has lasted weeks, months, or even years. This long-term burden of disease can be devastating to local economies and represents a significant health cost: CHIKV was responsible for 1386 to 1,081,962 nondiscounted years of life lost in 2005.[36] These values are now significant underestimations of the true health cost burden, given its expanding distribution to the Americas since those estimates were made.

Neurologic complications of acute CHIKV disease were observed with the 2006 outbreak on La Réunion Island, and include encephalitis[37] and Guillain-Barré syndrome.[38] These also were observed during CHIKV outbreaks in India.[39–41] Additional observations of severe manifestations of disease, including myocarditis and hepatitis,[37] have re-energized investigations of CHIKV disease pathogenesis.

CHIKV infection had not been associated with increased risk of mortality before 2006. During the outbreak on La Réunion Island, however, at least 213 people with CHIKV infection died. Investigators estimated that the case-fatality rate was approximately 1:1000, and observed that the fatalities occurred mainly in persons greater than or equal to 75 years of age.[42] During the 2006 outbreak of CHIKV infection in Ahmedebad, India, 18 of the 90 confirmed cases of CHIKV infection were fatal. Fifteen of the 18 deaths occurred in persons 60 years of age and older.[43] Autopsy of fatal cases of CHIKV infection in Colombia revealed evidence of hepatocellular coagulative necrosis, tubulointerstitial nephritis, and acute pericarditis.[44]

DIAGNOSTIC EVALUATION

Good laboratory testing services are important in the diagnosis of suspected CHIKV infections. Chikungunya fever can easily be confused at various stages of the disease with other arboviral infections, such as dengue and Zika virus. The clinical consequences of these three viruses are different, so specific diagnosis is important. Situations where laboratory test confirmation for presence or absence of infection are discussed next.

Testing sporadic cases of suspected arboviral infection can provide early warning that the virus is in the community. This can help public health and medical personnel prevent a possible epidemic. This is especially important if conditions conducive to an epidemic are present, such as during the rainy season when the mosquito population is high, and with open housing conditions that enhance exposure of an immunologically naive population to infection. Diagnosis of a significant number of cases early on to establish cause of an epidemic and the characteristic symptoms enables reliable clinical diagnosis with decreased need for laboratory confirmation if an epidemic occurs. Public health departments may occasionally perform epidemiologic testing for evidence of past CHIKV infection in a community so that the immunologic history and state of susceptibility in a population are determined to better approximate risk for an outbreak.

Focused and random spot testing is important during an epidemic to detect the entrance of a second arbovirus, such as DENV, entering the population during a

CHIKV outbreak. Focused testing is important in cases with serious underlying diseases and in cases with complications or a fatal outcome.

In the postoutbreak period, persons not previously tested for CHIKV should be tested for a recent or past infection as part of the work-up in patients presenting with new chronic arthritic problems including joint pain and swelling. During interepidemic periods, patients presenting with typical CHIKV symptoms should be tested for CHIKV and other arboviruses with similar symptoms.

Three tests for CHIKV are useful in various situations. For diagnostic confirmation of current and recent infection, a molecular test (typically polymerase chain reaction [PCR]) for the virus and an assay for the presence of specific IgM antibody are required. The most frequently needed assay is the CHIKV IgM antibody assay. Molecular testing for the presence of the virus is required in the early stages of disease. Virus is present in the blood at the time symptoms appear and PCR testing provides reliable detection for 5 days thereafter (6 days total). During that time only a molecular test for the virus should be ordered. As IgM production rises, by Days 7 to 9, viremia falls to PCR undetectable levels. During Days 5 to 9 when the viral load is waning and IgM has not reached its peak, it is necessary to order a molecular test for the virus and the IgM antibody assay to maintain good diagnostic sensitivity. After that, only the IgM test is required. After 14 to 21 days both the IgM and IgG test are positive, and the IgM test wanes over several weeks or months, whereas IgG remains for years as a good marker of past infection and immunologic protection, and also as an epidemiologic tool to determine seroprevalence in a population.

Using Grenada as a case study to illustrate the CHIKV diagnostic strategy, during the peak of the 2014 Grenada CHIKV epidemic, 112 samples from typical cases were tested by PCR and the IgM assay independent of the stage of disease. Although all 112 had classic symptoms, only 101 were found to be positive by at least one of these laboratory tests. In the 101 samples, IgM outperformed PCR: 92% were positive by IgM, 17% were positive by PCR, and 9% were positive by both. Reliance on PCR testing only is unlikely to accurately characterize incidence.[45–47]

Commercially available kits are of variable quality.[48] PCR assays with favorable performance characteristics are documented in the literature and becoming commercially available for clinical use in other countries; however, they can only be obtained for research use in the United States. Good immunoassays for CHIKV-specific IgM in patient serum are less reliable and available.[49–51] A partial list of sources for molecular viral assays and IgM assays to document CHIKV infection is given in **Table 1**. These sources can serve as starting points to explore test kit choices and capabilities in what it is hoped will be an expanding menu of kits for clinical testing.

Until reliable Food and Drug Administration–approved kits for molecular detection of virus and CHIKV antibodies are available, only the largest commercial and government reference laboratories should consider routine diagnostic testing for CHIKV. The Centers for Disease Control and Prevention has facilities for testing samples to establish transmission of CHIKV in the United States. Some city and state health departments and other government agencies also have this capability. Specific details related to collection and preserving serum for transportation to and testing at regional or national facilities can be found at: http://www.cdc.gov/chikungunya/hc/diagnostic.html.

If an epidemic should require greater testing capacity, the Centers for Disease Control and Prevention and similar agencies in other countries may implement Emergency Use Authorization for diagnostic tools for CHIKV that could be distributed to qualified clinical laboratories that demonstrate proficiency with the assays by successfully testing verification panels for each assay.

Table 1
Sources for commercially available kits for detection of chikungunya virus and IgM and IgG antibody in serum[a]

Company	Address	Product	Comments
Altona Diagnostics, GmbH	Mörkenstrasse 12 22767 Hamburg, Germany	RealStar Chikungunya RT-PCR Kit	
Liferiver Bio-Tech	9855 Towne Center Drive, San Diego, CA 92121	Chikungunya Virus Real Time RT-PCR Kit	CE approved
Primerdesign, Ltd	York House School Lane, Chandler's Ford, United Kingdom, SO53 4DG	Chikungunya Virus Real Time RT-PCR Kit	Dengue, Zika, and chikungunya virus multiplex kit also available
GenWay Biotech, Inc	6777 Nancy Ridge Drive, San Diego, CA 92121	Real Time RT-PCR Kit IgM μ-capture ELISA IgG capture ELISA	Separate PCR kits formatted for two commonly used PCR instrument types Provides PCR and ELISA assays
Euroimmun AG	Seekamp 31, D-23560 Luebeck, Germany	IgM ELISA IgG ELISA	
IBL Tecan US, Inc	9401 Globe Center Drive, Suite 140, Morrisville, NC 27560	Chikungunya IgM μ-capture ELISA	Laboratory ELISA automation
NovaTec Immundiagnostica, GmbH	Waldstrasse 23 A6, Dietzenbach, 63128, Germany	IgG ELISA and an IgM μ-capture ELISA	

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FDA, Food and Drug Administration; RT, reverse transcriptase.

[a] Partial list of companies providing PCR and ELISA kits for detection of CHIKV or human antibody to the virus. None of these are FDA approved for use as diagnostic tests at this time but are considered in the Research Use Only category. One product is CE approved. These tests may prove to be useful adjuncts to assist in the development of future FDA-approved or validated assays.

If CHIKV infection becomes an annual endemic threat in certain regions, there will be a need for testing capability on a more local basis. It would be ideal to have CHIKV detection as part of a routine test panel and supported by the clinical laboratory testing industry. Encouraging research studies suggest that this will be possible, first for PCR and perhaps later for the IgM assays.[46,51,52]

TREATMENT

There is presently no licensed targeted therapy for acute CHIKV infection. Treatment is primarily supportive care and includes the use of analgesic and anti-inflammatory medication, rehydration, and rest. However, research to identify potential new antiviral therapies, or repurposing of existing compounds for treating CHIKV infection is ongoing (**Table 2**; reviewed in Ref.[53]). For example, chloroquine has in vitro activity against several viruses, and has been found to inhibit CHIKV replication in Vero cells.[54] However, it has

Table 2
Examples of investigational strategies under development for treatment of CHIKV

Therapeutic	Mechanism	Data	
		In Vitro	In Vivo
Chloroquine	Inhibits fusion of CHIKV E1 protein with the endosomal membrane	Inhibited CHIKV infection in Vero A cells[54]	No efficacy in clinical trials in patients infected with CHIKV[58]
siRNA targeting CHIKV genes	Inhibits protein synthesis	Inhibited CHIKV replication in Vero-E6 cells (>90%)[59]	Inhibited CHIKV replication in mice when administered 3 d postinfection[59]
Ribavirin	Inhibits viral genome replication by depleting guanosine triphosphate	Inhibited CHIKV replication in Vero cells Synergistic inhibitory effect in combination with IFN-α2b and doxycycline[60]	Reduced the viral load and inflammation in infected ICR mice when combined with doxycycline[57]
Favipiravir (T-705)	Inhibits viral genome replication	Inhibited CHIKV-induced cytopathic effect in Vero A cells[61]	Reduced mortality of infected AG129 mice and protected from neurologic disease[62]
Monoclonal antibody C9	Binds CHIKV E2 glycoprotein	Neutralized CHIKV pseudovirions in HEK293T cells and CHIKV in Vero cells[63]	100% survival of CHIKV-infected mice when given at 8 or 18 h postinfection[63]

Adapted from Abdelnabi R, Neyts J, Delang L. Towards antivirals against chikungunya virus. Antiviral Res 2015;121:62; with permission.

not been shown to have anti-CHIKV effects in vivo. Compounds that may interfere with viral entry, including phenothiazines[55] and flavaglines,[56] are being investigated as potential therapies. Ribavirin has been shown to have in vitro activity against CHIKV, and synergized with doxycycline to reduce viral load and inflammation in infected mice.[57]

Monoclonal antibodies to the CHIKV E1 and E2 proteins have been used to protect mice and nonhuman primates from developing CHIKV infection after viral challenge.[27,29] However, it is unclear whether passive immunization with monoclonal antibodies or hyperimmune serum can ameliorate symptomatology of CHIKV disease after infection has already been established.

SUMMARY

The past decade has seen explosive viral epidemics, from severe acute respiratory syndrome to Ebola to arboviruses including Zika and CHIKV. For some diseases, the human toll is acutely evident in the form of mortality or acute morbidity. For CHIKV and others, the long-term sequelae from infection are yet ill-defined. The prolonged debilitating arthralgia associated with CHIKV infection has tremendous potential for impacting the global economy, and should be considered when evaluating the human burden of disease and the allocation of resources. There is much still unknown about

CHIKV and the illnesses that it causes. Developing a better understanding of the pathogenesis of CHIKV infection is a priority and forms the basis for developing effective strategies at infection prevention and disease control.

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