

Control of immunopathology during chikungunya virus infection

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After several decades of epidemiologic silence, chikungunya virus (CHIKV) has recently re-emerged, causing explosive outbreaks and reaching the 5 continents. Transmitted through the bite of *Aedes* species mosquitoes, CHIKV is responsible for an acute febrile illness accompanied by several characteristic symptoms, including cutaneous rash, myalgia, and arthralgia, with the latter sometimes persisting for months or years. Although CHIKV has previously been known as a relatively benign disease, more recent epidemic events have brought waves of increased morbidity and fatality, leading it to become a serious public health problem. The host's immune response plays a crucial role in controlling the infection, but it might also contribute to the promotion of viral spread and immunopathology. This review focuses on the immune responses to CHIKV in human subjects with an emphasis on early antiviral immune responses. We assess recent developments in the understanding of their possible Janus-faced effects in the control of viral infection and pathogenesis. Although preventive vaccination and specific therapies are yet to be developed, exploring this interesting model of virus-host interactions might have a strong effect on the design of novel therapeutic options to minimize immunopathology without impairing beneficial host defenses. (*J Allergy Clin Immunol* 2015;135:846-55.)

Key words: Chikungunya virus, immune responses, immunotherapy, vaccination

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First identified in the southern province of Tanzania in 1952, chikungunya virus (CHIKV) is believed to have originated in Africa.^{1,2} Its name, *chikungunya*, is derived from a Makonde word meaning “that which bends up,” in reference to the frequent and debilitating joint pains associated with infection. CHIKV is a

Abbreviations used

CHIKV: Chikungunya virus
DC: Dendritic cell
DENV: Dengue virus
KIR: Killer cell immunoglobulin-like receptor
NK: Natural killer
nSP: Nonstructural protein
ORF: Open reading frame

member of the Togaviridae family and of the Old World alphavirus group. This group comprises 31 viruses, 7 of which can cause human joint disorders, namely CHIKV, Semliki Forest, O’Nyong-Nyong (Central Africa), Ross River and Barmah Forest (Australia and the Pacific), Sindbis (cosmopolitan), and Mayaro (South America and French Guyana) viruses.³

CHIKV is composed of a phospholipid envelope, a 60- to 70-nm-diameter capsid, and a single-strand positive-sense RNA of approximately 12 kb containing 2 open reading frames (ORFs). The ORF at the 5’ end encodes 4 nonstructural proteins (nsP1-nsP4), and the ORF at the 3’ end encodes the structural proteins capsid protein, envelope glycoproteins E1 and E2, and 2 small cleavage products (E3 and 6K).

The replication cycle of CHIKV is essentially similar to that of other alphaviruses.⁴ It begins with the attachment of E1 with unknown cellular receptors, which leads to the fusion of the viral particle to the host cell membrane. Of note, several receptors have been suggested, but to date, their putative roles have not been firmly established.^{5,6} After virus entry, the positive-sense genomic RNA acts directly as mRNA to produce nsPs, which are responsible for synthesis of further structural proteins required for viral encapsidation and budding.⁷

In Africa the virus is maintained in a sylvatic transmission cycle between nonhuman primates, bats, rodents, and other unidentified vertebrates; these animals have viremia but no pronounced physical manifestations. CHIKV infection is transmitted to the human population by *Aedes aegypti* or *Aedes africanus* mosquitoes and, since 2006, *Aedes albopictus* mosquitoes; this new vector has invaded most of the world over the past 30 years.⁸

This infection can be both endemic and epidemic. Schematically, the endemic form appears to be mainly rural, with continuous sporadic transmission to largely immune populations, and can be associated with small content outbreaks or isolated cases. During epidemics, CHIKV can circulate directly through a human-mosquito-human transmission cycle, without the need for animal reservoirs. Genetic changes in the E1 glycoprotein, which mediates viral entry at low pH and affects viral fusion, assembly,

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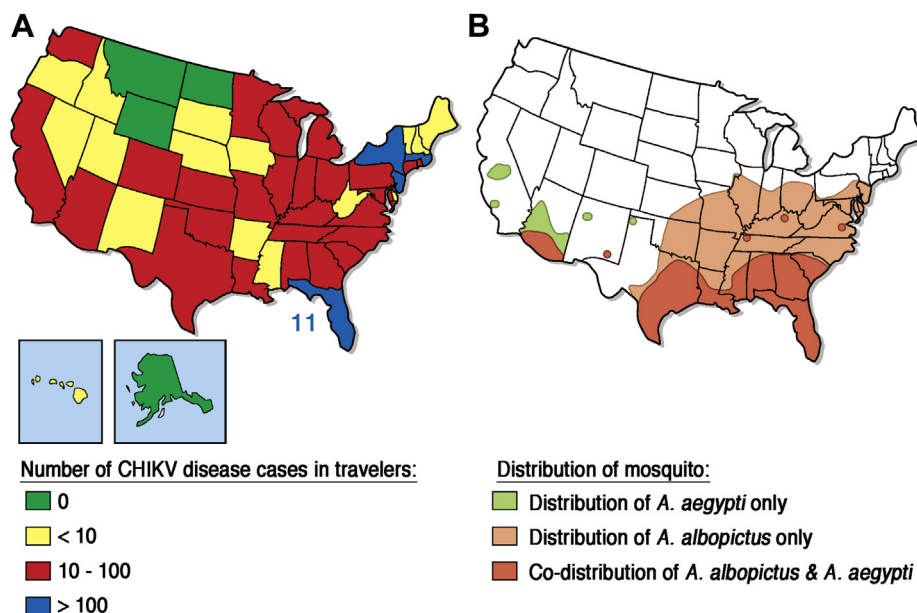


FIG 1. CHIKV infection cases and approximate distribution of *A. aegypti* and *A. albopictus* in the United States. **A**, Distribution of 2021 imported cases of CHIKV infection in the United States and 11 locally acquired cases reported in Florida reported to ArboNET in December 16, 2014. **B**, Distribution of *Aedes* species mosquito in the United States. This map was developed using currently available information from the National Center for Emerging and Zoonotic Infectious Diseases. Mosquito populations might be detected in areas not shaded on this map and might not be consistently found in all shaded areas.

and/or cell tropism, have enabled the virus to replicate efficiently in *A. albopictus*, consequently modifying the epidemiologic pattern of CHIKV. More recently, a second mutation was identified in the E2 gene E2-I211T, which was probably acquired by CHIKV strains around 2004-2005. This mutation has been shown to be a first necessary step to allow the subsequent E1-A226V substitution, providing a suitable background to allow CHIKV adaptation to *A. albopictus*.⁹ The combination of second-step adaptive mutations compels us to expect future emergence of CHIKV strains with even greater efficiency of transmission in the most endemic regions of the world.¹⁰

The most recent epidemic events mainly emerged in urban populations with weak specific immunity. In this setting the disease is characterized by an abrupt epidemic peak, with seroprevalence rates reaching up to 70% and then decreasing gradually as an increasing proportion of the population develops immunity.

CHIKV epidemics most certainly occurred before the virus was discovered, as suggested by descriptions of epidemic fevers accompanied by pronounced arthralgia. These fevers were attributed to other viral infections. On the basis of recent phylogenetic analyses, the evolutionary timescale of CHIKV suggests that sylvatic African CHIKV probably first emerged as a human pathogen in the 18th century and certainly re-emerged periodically with relatively low prevalence in Africa but also in Asia, with the earliest outbreak in the Philippines in 1954.¹¹ Since 2000, after an epidemiologic silent period, CHIKV prevalence has exploded worldwide. An urban epidemic of CHIKV was described in the Democratic Republic of Congo after an absence of about 40 years and then in Indonesia, causing 24 distinct outbreaks after a more than 20-year hiatus. In 2005, CHIKV emerged for the first time in the islands of the Indian Ocean. By June 2006, a third of the population of La Reunion was infected

by CHIKV; approximately 266,000 clinical cases and 203 deaths were associated with the disease.^{12,13} During this period, CHIKV was also reported in India,¹⁴ and several other countries in Southeast Asia, resulting in an estimated 1.9 million cases reported by the World Health Organization. Minor outbreaks have been observed in Africa until 2012.¹⁵⁻¹⁸ The first evidence of autochthonous CHIKV transmission in Europe was recorded in Italy in 2007¹⁹ and in the Americas in December 2013.²⁰⁻²² As of August 29, 2014, the Pan American Health Organization has recorded 776,000 cases and 152 deaths attributed to CHIKV infection in 33 countries and territories of the Americas (<http://www.paho.org/chikungunya>). The United States ArboNET, the national surveillance system for arthropod-borne diseases, reported 11 locally transmitted cases in Florida, as well as 3984 cases from Puerto Rico and the US Virgin Islands. The main CHIKV vectors are found to be increasingly present in temperate regions, such as Europe and the Americas. At the same time, the potential for viremic travelers returning from epidemic regions is on the increase. As an example, in the United States 109 imported cases of CHIKV were identified between 1995 and 2009, and 115 cases were identified between 2010 and 2013.^{23,24} As of December 16, 2014, 2021 cases were identified in 46 of 50 US states in travelers returning from the Americas ($n = 1989$), Asia ($n = 11$), or the Pacific Islands ($n = 10$; Fig 1, A). It is reasonable to augur that these patients could present sufficient levels of viremia to infect competent mosquitoes and act as Trojan horses for the introduction and spread of CHIKV infection in nonendemic areas. The risk of emergence of CHIKV in US territories is higher yet, considering the wide distribution of *A. aegypti* and *A. albopictus* in the country (Fig 1, B).

To date, CHIKV infection has been identified in nearly 80 countries across 5 continents and caused more than 6 million

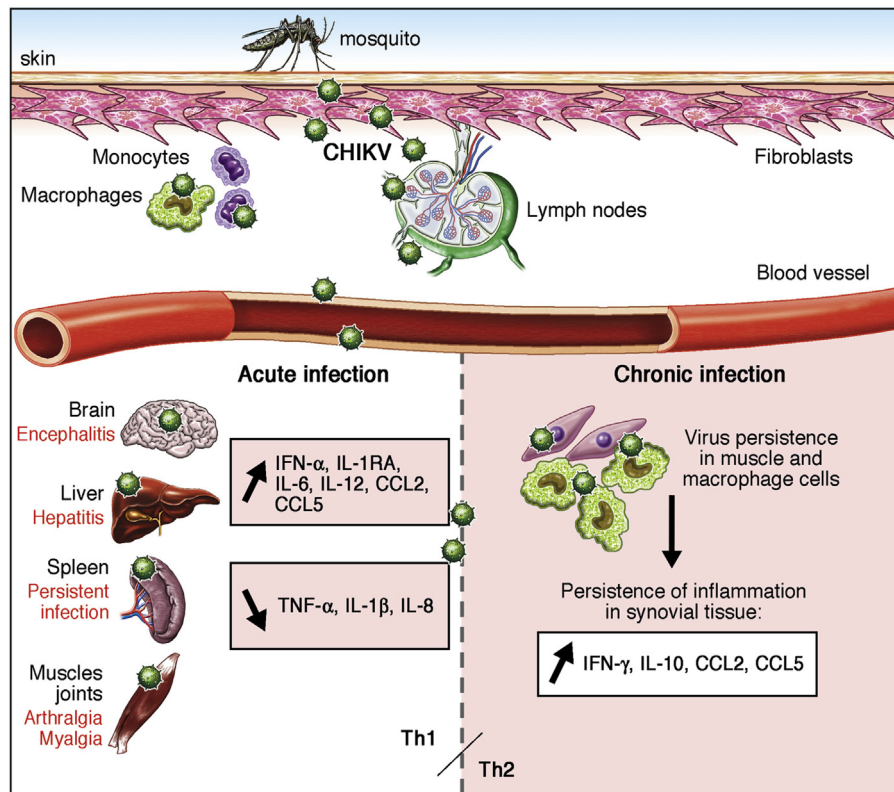


FIG 2. Viral dissemination, clinical manifestations, and chronic persistence in CHIKV-infected patients. CHIKV is transmitted through the bite of a female mosquito. The virus infects susceptible cells of the dermis, such as endothelial cells, fibroblasts, and macrophages, and replicates rapidly. Locally produced viral particles are transported through the circulatory system to secondary lymphoid organs and then disseminated to different organs, including the brain, spleen, liver, joints, and muscles. This acute phase of infection is associated with a high release of type 1 interferons, followed by upmodulation or downmodulation of many other cytokines, chemokines, and proinflammatory mediators. CHIKV can persist for weeks after primary infection in patients with a chronic disease. Infection of macrophages in the joints is accompanied by local and long-lasting inflammation of the synovial tissues.

confirmed cases. Considering the explosive nature of the epidemics in completely susceptible populations and the rapidly increasing counts, CHIKV has become a major public health issue. As such, in 2008, CHIKV was listed as a US National Institute of Allergy and Infectious Diseases category C priority pathogen. Intriguingly, outbreaks were mostly reported to be unpredictable, with an interval of several decades between major events. The precise factors of CHIKV emergence are unclear, but we can hypothesize that, in addition to ecologic and virus-linked factors, the immune status of the affected populations plays a key role both in the intensity and periodicity of recurrence.

RECENT PROGRESS IN UNDERSTANDING CHIKV PATHOGENESIS

What of the acute infection?

After intradermal inoculation by an infected female mosquito, CHIKV enters the subcutaneous capillaries, where it immediately starts to replicate within permissive cells of the skin, such as macrophages, fibroblasts, and endothelial cells (Fig 2). In a zebrafish model Palha et al²⁵ have shown that infected cells rapidly appear in various organs within a median of approximately 14 hours after infection. Although the host is mounting a response to control the virus in the dermis, the virus disseminates rapidly to the blood

circulatory system (Fig 2). During this silent incubation period, which lasts 3 to 7 days on average (range, 1-12 days), blood-circulating viral loads increase rapidly to reach tremendous concentrations detectable in the first few days of symptoms, sometimes reaching greater than 10^{10} copies/mL of plasma, which is uncommon in other arboviral diseases.²⁶ The peak viral titer was detected in the first 5 to 6 days after onset of symptoms in southern Thailand and Singapore in 2008^{26,27} but as early as in the first 1 to 3 days in patients from La Reunion Island in 2006.²⁸ As in other viral infections, the acute phase of CHIKV infection is accompanied by an early IFN- α response,^{29,30} which is detected as early as the first day of infection. IFN- α concentrations correlate with plasmatic viral load. In interferon response factor-deficient mice, it was demonstrated that inadequate IFN- α responses after CHIKV infection can be sufficient to induce hemorrhagic fever and shock,³¹ a finding that further highlights the importance of these cytokines in the pathology of CHIKV infection. Concurrently with the increase in viral load and IFN- α responses, a very high proportion of CHIKV-infected patients (72% to 97%) experience clinical symptoms compared with those with other arbovirus infections.³² Clinical onset is abrupt and most often characterized by high fever (typically $>39^{\circ}\text{C}$ [102°F]) associated with a wide range of other acute and nonspecific manifestations combining headache, myalgia, diarrhea, vomiting, abdominal pain, and rash. Intense

TABLE I. Clinical symptoms and laboratory findings of CHIKV infection

Clinical symptoms				
Major	Minor	Atypical		Laboratory findings
Fever	Arthritis	Bullous skin lesions	Myelitis	Lymphopenia
Abrupt onset	Conjunctivitis	Cranial nerve palsies	Myocarditis	Thrombocytopenia
Typically >39°C	Headache	Guillain-Barre	Nephritis	Creatinine +++
Joint pain	Myalgia	Hemorrhage	Uveitis	Transaminase +++
Severe and debilitating	Nausea	Hepatitis	Retinitis	
Multiple joints	Rash	Meningoencephalitis		
Bilateral and symmetric	Vomiting			

TABLE II. Clinical and laboratory features of CHIKV infection compared with those of DENV infection

	CHIKV	DENV
Clinical features		
Fever (>39°C)	+++	++
Arthralgia	+++	+/-
Arthritis	+	-
Headache	++	++
Rash	++	+
Myalgia	+	++
Hemorrhage	+/-	++
Shock	-	+
Laboratory features		
Lymphopenia	+++	++
Neutropenia	+	+++
Thrombocytopenia	+	+++
Hemoconcentration	-	+++

Symbols indicate the percentage of patients exhibiting each feature: +++, 70% to 100% of patients; ++, 40% to 69%; +, 10% to 39%; +/-, <10%; -, 0%.

polyarthralgia, the hallmark symptom of CHIKV infection, affects mainly the extremities (ankles, wrists, and phalanges) but can also affect the large joints (http://www.cdc.gov/chikungunya/pdfs/CHIKV_Clinicians.pdf, Table I).³³

CHIKV infection is often confused with dengue virus (DENV) infection. Both diseases present with high temperatures and myalgias in persons living in or returning from tropical areas and are transmitted by the same species of mosquitoes. Despite certain clinical similarities, the prevalence of specific symptoms might help with the initial differential diagnosis; prominent and prolonged arthralgias affecting multiple joints are more consistent with CHIKV, and hemorrhage is more common in cases of DENV infection (Table II). Other alphaviruses can also induce fever and arthralgias, and therefore the traveler's itinerary is important to consider while identifying probable cause (Table III). CHIKV infection is confirmed in the laboratory by means of viral isolation, detection of viral nucleic acid in serum samples with RT-PCR, or detection of an IgM antibody response (<http://www.cdc.gov/chikungunya/hc/diagnostic.html>, Table III).

Persisting symptoms seem to be a direct consequence of the viral infection and the associated proinflammatory immune response, as reported in different studies showing the early detection of various proinflammatory cytokines. Viral load has been linked to concentrations of IFN- α but also IL-1RA (IL-1 receptor antagonist), IL-6, IL-12, CCL2, and CXCL10.^{26,29,30,34,35} The T_H1 cytokine response is essential to promote immune cell activation but might also cause tissue injuries. Surprisingly, proinflammatory cytokines, such as

TNF- α , IL-1 β , and IL-8, were poorly expressed in the acute phase of CHIKV infection, and T_H2 (IL-4, IL-6, IL-10, and IL-13) cytokines were only expressed in a few CHIKV-infected patients (Fig 2).^{34,36} IFN- γ and IL-12 are known to act synergistically to promote innate immune cell activation, and levels both were highly increased in the majority of CHIKV-infected patients.^{30,34} However, results related to cytokines in patients with CHIKV infection are usually contradictory. Discrepancies between studies for some markers could reflect different genetic backgrounds, heterogeneous sanitary conditions, and patient management strategies, as well as differences in laboratory techniques. In an experimental infection of macaques with CHIKV, early production of IFN- α , IL-6, and CCL2, followed by IFN- γ , TNF- α , CCL3, and CCL4, was detected.³⁷

What of the chronic infection?

The acute signs and symptoms of CHIKV infection usually resolve within 1 to 3 weeks, but the arthralgia can persist for months or even years in some patients. This chronic phase, which is usually characterized by fluctuations in intensity and relapses, is generally less severe than the acute phase, but many patients still experience a pronounced loss in movement and quality of life. The proportion of patients with chronic signs attributable to CHIKV 3 months after infection differed between outbreaks. In Singapore 13% of infected patients still had chronic arthralgia, whereas nearly half of the patients with persistent rheumatic pain were impaired in carrying out daily or household activities.²⁶ These manifestations were independently associated with older age, severe initial pain, and the presence of comorbid osteoarthritis.³⁸ Children are also at risk for severe manifestations of the disease with common clinical features, such as chronic swollen ankles or wrists.^{39,40} It is plausible that CHIKV persisting in immune-privileged niches directly inflicts damage to the synovial tissue. Indeed, Hoarau et al²⁹ provided evidence of persistence of viral antigen and RNA in synovial tissue in a patient with chronic arthralgia for as long as 18 months after CHIKV infection, and viral persistence was associated with expression of IFN- α , IL-10, and CCL2 and proinflammatory cytokines. This is consistent with findings in macaques showing evidence of viral persistence in lymphoid tissues (Fig 2).³⁷ Regulatory mechanisms silencing the inflammatory response seem to be required to prevent establishment of a chronic disease, and it seems that a dysregulation of inflammation during the acute and convalescent phases or the absence of an anti-inflammatory response leads to development of chronic arthralgia or arthritis. Supporting this hypothesis, Hoarau et al²⁹ detected CHIKV and various markers of inflammation (IFN- α , IL-6, IL-8, and

TABLE III. Diagnostic criteria for CHIKV infection

		Actions
1: Clinical criteria		
Residing or visiting an epidemic area within 15 d before onset of symptoms (high fever and/or severe arthralgia)		Determination of clinical symptoms (Table I)
2: Differential diagnosis		
CHIKV coexists with other infectious diseases, such as dengue, other alphavirus infections, or endemic malaria		Comparative analysis of clinical and laboratory features (Table II)
3: Laboratory criteria after onset of symptoms		
Confirmation of infection through:		Optimal timing after illness onset:
Isolation of virus		≤3 d
Presence of viral RNA (PCR)		≤8 d
Detection of specific antibodies (IgM)		≥4 d

CCL2) in the synovium of a patient with chronic pain that were absent from the synovium of patients who fully recovered. In addition to the role of inflammatory cytokines, the possibility of the virus directly hijacking the resident immune cells, locally disrupting the immune response, and causing tissue damage also needs to be investigated ([Fig 2](#)).

What of the severe forms?

Historically, CHIKV was considered self-limiting and nonfatal. However, neurologic complications were observed in patients who had a medical history of epilepsy, heavy alcohol consumption, or both, and a variety of other severe clinical symptoms have been reported, such as myocarditis, pneumonia, lymphadenopathy, hepatitis, and pancreatitis ([Table I](#)). Overall, it was estimated that severe cases occurred in only 0.3% of all symptomatic patients of the 2006 Reunion Island outbreak, and the fatality rate was estimated at 1 in 1000.⁴¹ A direct causal link between death and CHIKV infection had not been demonstrated until 2006, during the epidemic in the Indian Ocean, where an important number of deaths were directly attributed to CHIKV infection. This trend was confirmed in the most recent outbreak in the Americas, with a total of 152 deaths reported in August 2014. With a rate of mother/child transmission of about 50%,⁴² neonates exposed in utero are particularly at risk, as are adults with underlying medical conditions (eg, hypertension, diabetes, or cardiovascular disease) and the elderly (>65 years). Common causes of death are heart and multiple organ failures, hepatitis, and encephalitis, yet the underlying mechanisms of pathogenesis are unclear.^{41,43} A series of biological abnormalities or immunologic markers are associated with disease severity, such as increased liver enzymes, creatinine, and C-reactive protein levels; hypocalcemia; increased IL-1 β and IL-6 levels; or decreased CCL5 levels.^{29,30,34} Furthermore, an association between viral load and acute severe illness is controversial, although clearly reported in macaques.³⁷ The potential effects of new mutations found in CHIKV strains on viral replication and pathogenicity, as well as their clinical repercussions, need to be thoroughly investigated.

The identification of selective profiles of cytokines and other molecular markers in the very early stages of CHIKV infection would constitute a powerful prognostic tool and could be important for early recognition and management of patients most at risk of persistent or severe symptoms. The persistent nature of CHIKV and its incidence in chronic or severe forms need

to be thoroughly investigated in the hope of counteracting the debilitating and lethal effects of this infection.

RECENT PROGRESS IN IMMUNE CONTROL OF CHIKV PATHOGENESIS

The outcome of viral infections is determined by a series of intricate interactions between the pathogen and the host's immune system. On the one hand, coevolution of the virus and its preferred host has led to the development of specialized immune recognition mechanisms for detecting viral infection and of unique mechanisms for restricting viral replication. On the other hand, much like many other pathogens, CHIKV has evolved to escape the cellular immune responses by hijacking antiviral pathways. The CHIKV protein nsP2, for example, has been shown to induce the degradation of a subunit of the cellular RNA polymerase II, which is indispensable to cellular transcription, therefore blocking the activation of cellular genes, including those involved in the antiviral response.⁴⁴

The cellular immune responses to CHIKV infection have been relatively understudied. Infection is often associated with profound lymphopenia,²⁹ and CHIKV does not replicate in lymphocytes, monocytes, or monocyte-derived dendritic cells (DCs).⁴⁵

What of the innate immunity?

Natural killer (NK) cells constitute the proverbial first line of defense against a variety of viral infections. They express a balance of inhibitory and activating signals that enable them to detect and lyse virus-infected target cells while sparing normal cells.⁴⁶ Growing evidence shows the importance of these early responders in the control of CHIKV infection. In the course of infection, NK cells are spared from the profound lymphopenia and display an activated profile.^{29,47} Moreover CHIKV infection skews the NK cell receptor repertoire toward the activating CD94/NKG2C receptor, which is usually very poorly or not at all expressed on NK cells of healthy donors ([Fig 3](#)).⁴⁷ Other viral infections were also reported to have an effect on the NKG2C⁺ NK cell subset, such as cytomegalovirus, hantavirus, and HIV-1 infections.⁴⁸ With CHIKV, NKG2C⁺ NK cells are more potent at promoting cytotoxicity than producing IFN- γ ,⁴⁷ suggesting a prominent role in the control and destruction of virus-infected cells. Paradoxically, perhaps, the lack of IFN- γ in the microenvironment could alter or delay initiation of the adaptive immune

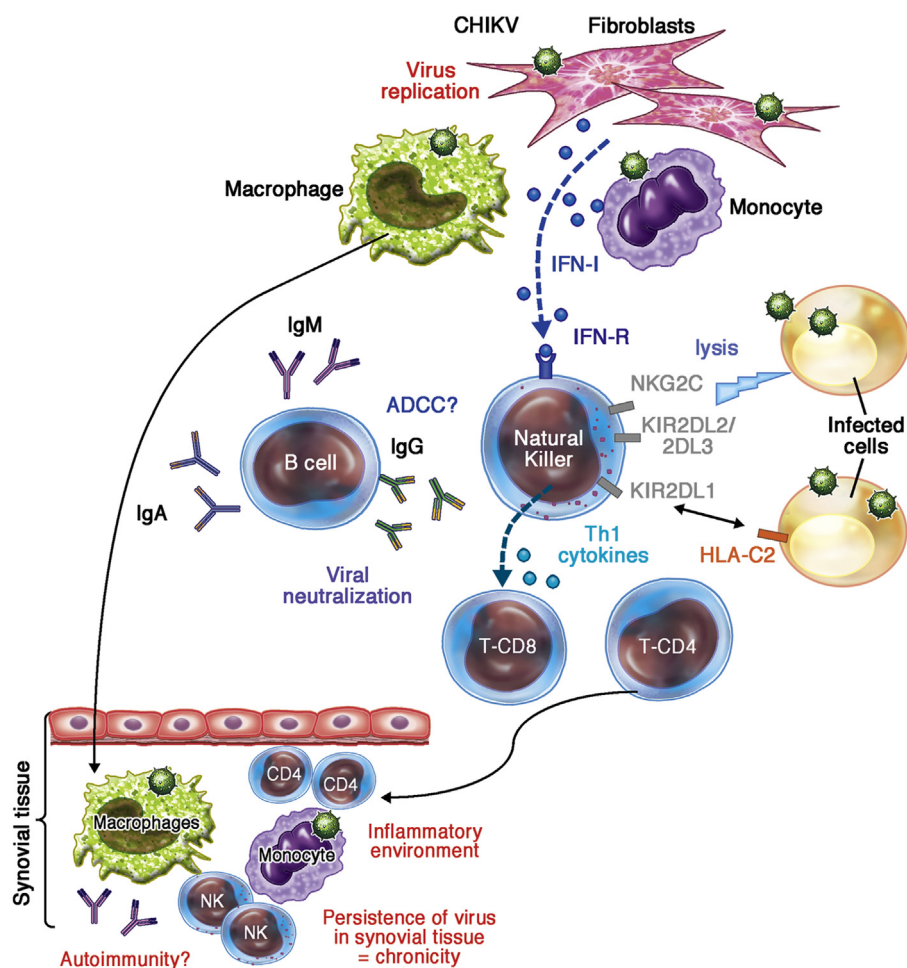


FIG 3. Model of immune activation in patients with CHIKV infection. CHIKV infection triggers activation of an innate immune response primed by strong production of type 1 interferon (*IFN-I*), which signals through its receptor (*IFN-I-R*), which is present on most cells, including NK cells. NK cells are major actors of innate immunity able to target CHIKV-infected cells through a NKG2C/KIR-HLA class I-dependent cytotoxic mechanism. They also participate in the recruitment of T lymphocytes through production of T_H1 cytokines. Evidence of CHIKV-specific adaptive immunity is mostly observed after viral clearance. Specific anti-CHIKV antibodies are produced and contribute to controlling the virus through viral neutralization and/or antibody-dependent cell-mediated cytotoxicity (undetermined to date). T lymphocytes and infected macrophages might contribute to the persistence of the virus and the development of localized inflammation in the synovial tissues of chronically infected patients.

response and be associated with a delay in resolution of persistent symptoms. Whether NKG2C, other activating receptors, or both participate in processes leading to the control of CHIKV infection awaits further investigation. NK cells also express killer cell immunoglobulin-like receptors (KIRs), which are encoded by a family of highly polymorphic genes. It has become increasingly clear that the strength of the interaction between KIRs and their HLA ligands has functional significance and can influence the susceptibility to and outcomes of various infectious diseases.⁴⁹ We have recently shown that the genetic combination of KIR2DL1 and its cognate HLA-C2 ligand is significantly associated with susceptibility to CHIKV infection (Fig 3).⁵⁰ In all, NK cells seem to be beneficial in the acute phase of CHIKV infection. Nevertheless, *a posteriori*, NK cells could contribute to immunopathologic mechanisms by infiltrating synovial tissues and maintaining an inflammatory environment that could contribute to the development of arthralgic symptoms.²⁹

Unlike NK cells, circulating monocytes and DCs do not display an activated phenotype during acute CHIKV infection.²⁹ However, Long et al⁵¹ have recently suggested that a modulation of expression of the DC immunoreceptor, which is known to be associated with rheumatoid arthritis, might play a major role in regulating inflammatory responses during the acute stages of disease. In addition, there is compelling evidence showing that monocytes/macrophages play an important role in the development of CHIKV-induced pathology; they act as a cellular reservoir for CHIKV persistence and seem to participate in the control of apoptosis and $IFN-\alpha$ signaling.^{37,52} Furthermore, infected monocytes/macrophages migrate to the synovial tissues, where they contribute to the inflammatory process. This could explain the persistence of joint symptoms despite the short duration of viremia (see above).⁵³ In a similar way infected monocytes/macrophages might be the main cells responsible for viral dissemination to other sanctuary body sites, such as the

TABLE IV. Recent success of approaches to a CHIKV vaccine

Type of vaccine	Description	Current status	Reference
Attenuated	CHIKV from the LR2006-OPY1 strain attenuated by an EMCV/IRES	In mice	Plante et al ⁷³
	CHIKV from the LR2006-OPY1 strain attenuated by a large deletion in nsP3 ($\Delta 5\text{nsP3}$)	In macaques	Hallengård et al ⁷⁴
MVA	Recombinant MVA expressing the CHIKV envelope polyprotein cassette E3E26KE1 from the CHIKV-S27 strain	In mice	van den Doel et al ⁷⁵
	Recombinant MVA encoding the capsid and E1-E3 from the CHIKV strain LR2006-OPY1 produced in chicken fibroblast DF-1 cell line	In macaques	García-Arriaza et al ⁷⁶
DNA	DNA encoding E1-E3 and the capsid from the CHIKV strain PENN	In macaques	Mallilankaraman et al ⁷⁷
	DNA replicon (DREP) encoding nsP1-nsP4 and E1-E3 from the CHIKV strain LR2006-OPY1	In macaques	Hallengård et al ⁷⁴
VLP	VLP composed of E1-E3, capsid and 6K proteins from the CHIKV LR2006-OPY1 and 37997 strains	In macaques	Akahata et al ⁷⁸
	VLP composed of E1, E3, and capsid proteins from the CHIKV 37997 strain produced in human embryonic kidney VRC293 cells (VRC-CHKVLP-059-00-VP)	Clinical trial in humans	Chang et al ⁷⁹

EMCV, Encephalomyocarditis virus; IRES, internal ribosomal entry site; MVA, modified vaccinia ankara; VLP, virus-like particles.

nervous system or synovial fluids, and thus might contribute to the development of severe clinical manifestations (Fig 3). Consistently, Labadie et al³⁷ have shown in an experimental macaque model that macrophages extensively infiltrate the lymphoid and liver tissues of all animals early after infection and in many cases remain in these sites for at least 6 months after virus inoculation.

What of the adaptive immunity?

Natural anti-CHIKV antibodies of IgM and IgG isotypes have been detected in patients at different stages of infection. Generally, IgM is detectable 2 to 3 days after the onset of symptoms and persists from several weeks to a year after resolution. CHIKV-specific IgG appears soon after (10-13 days after infection) and persists at high titers for years.⁵⁴ The persistence of IgM for months after the initial infection has been observed in patients with several other alphavirus infections and might be related to viral persistence through poorly understood mechanisms. Through a rapid and strong seroconversion, CHIKV infection seems to elicit long-lasting protective immunity (Fig 3). Anti-CHIKV antibodies are capable of providing early protection and controlling dissemination of the virus through direct neutralization and activation of the complement system.^{55,56} Her et al⁵⁷ have recently shown that sensing of CHIKV infection by Toll-like receptor 3 prompts development of the humoral response. Studies with human plasma obtained at different time points after infection have shown that the N-terminal region of the E2 glycoprotein elicits a long-lasting anti-CHIKV antibody response,⁵⁵ although the antibody recognition of E2 changes throughout the course of infection in experimentally infected macaques.⁵⁸ This suggests that differential induction of neutralizing antibodies against exposed or hidden B-cell epitopes could contribute to antibody-mediated clearance during the entire course of the disease. Neutralizing antibodies seem to be predominantly of the IgG₃ subtype, and therefore the absence of early CHIKV-specific IgG₃ might serve as a specific marker of patients with increased risk of disease.⁵⁹ Neutralizing antibodies to CHIKV generated early during infection are not associated with the development of typical symptoms, such as fever and joint pain,⁶⁰ and confer effective cross-protection against secondary CHIKV infections by different viral

strains. This is of particular interest considering that neutralizing antibodies persist for decades after initial infection in more than one third of the exposed population.⁶¹

Our current understanding of T-cell involvement during CHIKV infection is in its nascent stage. Very early after infection, frequencies and absolute values of T cells were found to be significantly lower in CHIKV-infected patients than in healthy donors. This was a transient event, and T-cell counts returned to baseline levels after a 2-month period.⁴⁷ A significant fraction of the remaining peripheral T cells were activated and expressed the surface death receptor CD95, and it has been hypothesized that overstimulation of T cells and CD95-dependent apoptosis of CD4⁺ T cells could, at least in part, account for the early lymphopenia.^{29,30} Although CD8⁺ T cells seem to be preferentially activated in the first few days of infection, a switch from CD8⁺ to CD4⁺ T-cell activation occurs in the later stages of acute infection (Fig 3). Studying T-cell immunoreactivity against CHIKV, Hoarau et al⁶² have shown that during the chronic phase of the disease, the IFN- γ T-cell response was mainly driven by CD8⁺ T cells. CD4⁺ and CD8⁺ T cells were found to infiltrate inflamed joints of several mouse models of CHIKV infection,⁶³ whereas further animal studies have suggested that CD4⁺, but not CD8⁺, T cells play a major role in mediating the severity of joint inflammation,⁶⁴ as previously described in patients with autoimmune arthritis.⁶⁵

It is important to note that after CHIKV infection, almost all patients had anti-CHIKV T-cell immunity, but surprisingly, only 20% of patients conserved memory T cells after 1 to 2 years after infection in a cohort of convalescents and chronically affected patients from the La Reunion outbreak.⁶² These findings need to be taken in account while developing vaccines.

Overall, many questions remain as to the complex interplay between CHIKV and the host's immune system. Between immune protection and immunopathogenesis, what role do NK cells play? Although IgG is produced early after infection, does NK cell antibody-dependent cell-mediated cytotoxicity participate in the destruction of infected cells? Considering that CHIKV does not directly target lymphocytes, which mechanisms govern the lymphocyte depletion? Of B-cell and T-cell memory, which long-term cellular immunity confers optimal protection against future infections? What are the major immunologic factors involved in CHIKV chronicity, and do regulatory T cells play a

part in this process? And what is the involvement of cytokine deregulation as opposed to direct viral cytotoxicity in the targeting of CHIKV-infected or bystander noninfected cells? Answers to these questions will surely provide important clues for the development of new antiviral therapies.

RECENT PROGRESS IN THERAPEUTIC AND VACCINE DEVELOPMENT

The recent explosive dissemination of CHIKV throughout immunologically naive countries stresses the need for appropriate treatments and preventive strategies. To date, there is no vaccine against CHIKV infection licensed for human use, and therapy for CHIKV infection is still mainly limited to supportive care. The World Health Organization only recommends a “treatment directed primarily at relieving the symptoms, including the joint pain using anti-pyretics, optimal analgesics and fluids. Corticosteroids and anti-rheumatic drugs (mostly methotrexate) can be administered to the patients with chronic forms of the disease and symptoms of polyarthritis.” It is our purpose here to focus on the more recent immunotherapeutic and vaccine perspectives.

What of immunotherapy?

Passive immunotherapy has been used for more than a century to treat various viral infectious diseases, including alphavirus infections. Human polyvalent immunoglobulins purified from CHIKV-convalescent plasma exhibit a potent dose-dependent neutralizing effect in a neonatal mouse model of CHIKV infection.⁶⁶ This strategy might prove to be efficient in both the prevention and treatment of patients exposed to CHIKV and could be of particular interest for patients at risk of a severe infection, such as neonates born to viremic mothers and adults with chronic underlying medical conditions.⁶⁶ A phase I clinical trial ongoing in Pointe-à-Pitre hospital (Guadeloupe, France) aims to determine the safety of intravenous administration of anti-CHIKV hyperimmune human immunoglobulins to exposed neonates ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT02230163) Identifier: NCT02230163). As a potential alternative, human recombinant mAbs exhibited strong and specific neutralizing activity *in vitro* when tested against a wide variety of CHIKV strains and were able to significantly delay the CHIKV-driven lethality in mice.⁶⁷⁻⁶⁹

The mechanisms by which CHIKV causes symptoms are poorly understood; however, the inflammatory response has been shown to be key to disease development (see above). As such, therapies aiming to modulate inflammation are under consideration to be used as treatment, particularly to decrease long-term chronic manifestations. For instance, an inhibitor of CCL2 (called Bindarit [Abcam, Cambridge, United Kingdom]) was shown to be a potent anti-inflammatory drug in a mouse model of CHIKV infection and in treating arthritis resulting from infection with the related alphavirus Ross River virus.^{70,71}

What of vaccines?

Infection with CHIKV induces long-standing protective immunity. In animal models cross-protection is conferred against different isolates of CHIKV and even toward other alphaviruses. Based on these observations, efficient vaccination would be highly beneficial. The first CHIKV vaccine was described in

1972.⁷² Many potential vaccine candidates have been tested since, and several are currently under development (Table IV).⁷³⁻⁷⁹

Interestingly, the US Army Medical Research Institute of Infectious Diseases tested the vaccination potential of a live attenuated strain 181/clone25 in volunteers, which led to high seroconversion rates and neutralizing antibody titers.⁸⁰ Although initially promising, development of this vaccine was halted in 2003, and it was recently shown that the 181/clone25 strain reverts in vaccinated mice, suggesting genetic instability.⁸¹ More recently, a promising virus-like particle-based vaccine derived from the CHIKV strain 37997 (VRC-CHIKVLP059-00-VP) induced the production of high-titered neutralizing antibodies in monkeys and protected the animals against viremia after challenge.⁷⁸ This vaccine has recently been tested in a phase I clinical trial in 25 healthy participants ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT01489358) identifier: NCT01489358) and was shown to be safe, well tolerated, and immunogenic,⁷⁹ suggesting a protective effect that needs to be confirmed through larger studies in populations at risk of CHIKV infection.

The recent massive outbreaks indicate that CHIKV will probably continue to extend dramatically its geographic range and effect on public health. These most promising therapeutic and preventive approaches need to be urgently tested and developed. However, it is important to note that CHIKV could escape the immune system, as suggested by the long-term persistence of CHIKV in synovial macrophages and the long-lasting production of CHIKV-specific IgM antibodies. To date, it is unclear whether CHIKV is capable of escaping neutralizing antibody responses by undergoing genetic mutation. Rare investigations suggest that neutralization-escaping viruses could be selected during combination therapy with neutralizing mAbs and associated with a partial decrease in their efficacy.^{82,83} In the context of the development of future immunotherapies and vaccines, it will be very important to pursue these studies.

CONCLUDING COMMENTS

Many lessons can be drawn from the recent outbreaks of CHIKV. First, the clinical manifestations associated with CHIKV infection are highly variable and might be more severe than previously reported, particularly within “naïve” populations.

Second, economic development does not protect countries from this infection. On the contrary, a modern lifestyle might amplify an epidemic through travel, and the prevalence of underlying medical conditions might increase the frequency of severe infections.⁸⁴

Third, occurrence of CHIKV infections fluctuates with climate. CHIKV appears seasonally in highly endemic areas, and climate changes seem to be of critical importance for the survival and spread of *Aedes* species mosquitoes, as well as the biting rate and incubation time within these vectors of disease. Considering the numerous environmental, viral, and human-related factors, it is difficult to predict how this emerging virus will behave in relatively new and uncharted territories.^{8,85} However, the recent urban transmissions into new populations in the Americas, Europe, and Africa provide us with the opportunities to improve our knowledge of the biology, epidemiology, and immunology of CHIKV infection.

Long-lasting arthralgic manifestations, the hallmark symptom of CHIKV infection, cause disability and handicap in the daily life

of a rapidly growing number of patients. Although the social and economic effects of having sometimes up to 60% of the population handicapped is immense, the pathogenic events leading to persistent pain and inflammation of the joints after infection are unclear. It is likely that CHIKV, persisting in immunoprivileged niches, directly induces synovial tissue damage. However, these viral sanctuaries raise a number of unresolved questions relating to the reactivation of viral production, the evasion of the immune antiviral response, the role of uncontrolled inflammation, or the possible induction of autoimmunity caused by cross-reactivity between viral and host antigens.

CHIKV circulates in regions that are prone to many other endemic diseases, such as DENV, West Nile virus, yellow fever, or malaria, for which *Aedes* species mosquitoes are also potent vectors. This considerable geographic and epidemiologic overlap has brought attention to the increasing challenge of coinfections. Thus far, several cases of CHIKV/DENV and CHIKV/malaria coinfections have been reported.^{16,86} Little to nothing is known in regard to the host's immune responses or to the subsequent clinical manifestations and outcome of patients coinfecting by vector-borne pathogens. Understanding the complex interactions between CHIKV and an infected host, as well as the interactions with other pathogens, is ever more important. The threat of coinfections together with the globalization of CHIKV epidemics and the unprecedented increase in magnitude in morbidity and mortality in the most recent outbreaks should prompt implementation of preventive measures and development of specific treatments to thwart future epidemics of CHIKV.

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