Chikungunya virus: Update on molecular biology, epidemiology and current strategies

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Abstract

Chikungunya virus (CHIKV), an arthropod-borne virus, is the aetiological agent of a disease characterized by several aspecific symptoms including fever, myalgia and arthralgia. The virus is primarily transmitted to humans through the bite of an infected mosquito of *Aedes* genus. This virus was first isolated in Tanzania in 1953, from which it spread to other African countries, Asia, Northern and Southern America, Europe and Oceania. Today, many human cases of CHIKV infection have been identified. The diffusion of CHIKV across the world, including Italy, is due to multiple factors amongst which the wide distribution of its vectors and high transmission efficiency play a crucial role. Currently, there are no specific treatments and effective vaccines against CHIKV; indeed, available therapies allow symptoms mitigation and some promising vaccines are undergoing clinical trials. The purpose of this review is to offer an updated picture of CHIKV molecular biology, epidemiology and vector distribution, clinical features and strategies for infection prevention and treatment.

Introduction

Chikungunya virus (CHIKV) is an arthropod-borne virus (arbovirus) included in the Alphavirus genus of the *Togaviridae* family.[1] It is well estabilished that CHIKV is the aetiological agent of a disease characterized by fever, myalgia, arthralgia, headaches, skin rash and joint swelling.[2] The virus is transmitted to humans by mosquito vectors belonging to *Aedes* genus; the high transmission efficiency promotes disease outbreaks even across urban environments. The origin of the term *chikungunya* comes from the African word *kungunyala,* literarily meaning *to be contorted*, and it refers to the typical patients' posture resulting from severe muscular and joint pain.[3,4] CHIKV was first isolated during an epidemic in Tanzania, in 1952-1953. However, it has been suggested that CHIKV outbreaks had already happened in Africa, Asia and America before this date and were generally mistaken for dengue epidemics.[5,6] In fact, as these viruses can cause similar clinical syndromes, retrospective studies suggested that they may have been confused in the past, especially in the regions in which they are both endemic. Since its discovery, the virus has spread, from Africa and Asia, where it caused sporadic outbreaks, particularly to the islands of the Indian Ocean and to Southeastern Asia, until it reached Europe (Italy, France and Spain) and the American continent,[7] as shown in Figure 1.

In 1999-2000, a large Chikungunya epidemic occurred in Congo.[8] In 2005, an epidemic event interested the islands of the Indian Ocean, such as La Réunion. During the same period, numerous cases in Europe have been reported as imported cases and associated with this outbreak. Another outbreak interested Gabon in 2007; while 1.5 million cases occurred in India.[9]

To date, millions of human CHIKV infections have been recorded all over the world.[10] In recent years, an ever more tangible threat to human health is represented by the re-emergence of infections caused by arboviruses, including Chikungunya.

Classification

Alphavirus and Rubivirus are the two genera constituing the *Togaviridae* family. The Alphavirus genus comprises 29 species distributed worldwide and able to cause disease in humans and animals.[11-32] (Table 1).

The need of specific vectors and reservoirs and particular ecological conditions influence the geographic distribution of the single species.

Alphaviruses are typically transmitted to susceptible hosts by an arthropod vector. In certain cases, virus-vector interactions may be highly specific, with only one vector type being capable of viral transmission.

Among Alphaviruses, nine are of medical and economic interest: CHIKV, O'nyong-nyong virus, Sindbis virus, Semliki Forest virus, Venezuelan Equine Encephalitis virus, Eastern Equine Encephalitis virus, Western Equine Encephalitis virus and Ross River virus.[32]

The available classification includes four distinct CHIKV genotypes, accordingly to the E1 gene sequence and viralgenome: i) East/Central/South African (ECSA); ii) Indian Ocean Lineage (IOL); iii) West African and iv) Asian.[33]

Two vectors, *Aedes aegypti* and *Aedes albopictus*, have been reported to be involved in CHIKV spread in large epidemic events. The first one is more common in tropical and subtropical regions, while *A. albopictus* is widespread in Africa, Asia, America and Europe.[7] CHIKV distribution corresponds to that of its specific mosquito vectors.

Virion structure and genomic architecture

CHIKV is an enveloped virus with an icosahedral nucleocapsid enclosing the viral genome. Mature viral particles have a diameter of about 700Å.[34] CHIKV presents a positive single-stranded RNA genome [(+)ssRNA] of about 11.8Kb.[35] The viral genome is capped and polyadenylated and includes two open reading frames encoding structural and non-structural proteins,[36] as shown in Figure 2. The large 5' Open Reading Frame 1 (ORF1), that extends over two-thirds of the viral genome, encodes for four non-structural proteins (nsP1, nsP2, nsP3 and nsP4) playing a crucial role in different functions including RNA capping, replication and protein cleavage.[37] ORF1 is translated into a single polyprotein which is

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then cleaved to produce the mature proteins.

The nsP1 presents RNA capping activity, nsP2 acts as an helicase and protease enzyme, nsP3 is crucial for genome synthesis and nsP4 is a RNA-dependent RNA polymerase.[36]

An untranslated junction-region links the ORF1 with the ORF2. The ORF2, placed at the 3' end of the viral genome, encodes for the viral structural proteins C1, E1, E2, E3 and 6K;[37] these proteins are translated as a large precursor polyprotein that is subsequently cleaved by proteases into the different proteins.[38]

The central core of the virus consist of about 240 copies of the capsid protein C1 associated with the viral genome to form an organized icosahedral nucleocapsid of about 400 Å in diameter.[39]

The nucleocapsid core is surrounded by a lipid envelope rich in viral spikes, whose packing differs between immature and mature particles.[39] These membrane-anchored structures are triplets of E1/E2 heterodimer organized in T=4 quasi-symmetry.[39]

The E2 and E3 proteins originate from a common precursor named p62. Together with E1, this protein is able to form the viral spikes in the endoplasmic reticulum. P62 cleavage in E2 and E3 proteins occurs during the transition from the progressively more acidic Golgi network to the early endosome, characterized by acidic pH, to the plasma membrane with neutral pH.[39]

E1 glycoprotein is a 442 aa protein organized in three β-sheet-rich domains respectively named I, II and III. Thanks to a fusion-loop located at the tip of the domain II, this glycoprotein plays a fundamental role in membrane fusion.[39,40]

E2 is a 423 aa glycoprotein composed by three immunoglobulin-like domains called A, B and C, connected by a β-ribbon. This CHIKV protein plays a major role in

Table 1. Alphaviruses: abbreviations, vectors, vertebrate hosts and main clinical features.

	Virus	Arthropod vector	Vertebrate host	Geographical distribution	Disease*	Source
1	BFV	*Culex annulirostris*	Human	Australia (Queensland, Victoria, New South Wales)	Arthralgia, rash	Flexman *et al.* (1998),[26] Jacups *et al.* (2008)[18]
2	CHIKV	*Aedes* spp. (*A. albopictus, A. aegypti*)	Human	Africa (including Southern Tanzania), America, India, Europe (including North- Eastern Italy)	Arthalgia, arthritis, encephalitis	Schwartz *et al.* (2010)[27]
3	EEEV	*Culis etamelanura,* occasionally *Coquillettidia perturbans and Aedes canadensis*	Horse, Human	Eastern USA (including North Carolina), Caribbean, South America	Encephalitis	Del Piero *et al.* (2001),[14] Beckwith *et al.* (2002)[12]
4	EVEV	*Culex cedecei*	Human	USA (Florida)	Encephalitis	Coffey *et al.* (2006)[13]
5	GETV	*Culex gelidus*	Human, Pig, Horse	Asia (including Malaysia), Northern Australia	Rash	Nemoto *et al.* (2015)[22]
6	HJV	*Culis etamelanura*	Equine, Human	North and South America	Encephalitis	Allison *et al.* (2009)[11]
7	MAYV	*Aedes aegypti*, *Culex quinquefasciatus*	Human	South America, (including Trinidad, Brazil)	Arthritis, rash	Long *et al.* (2011)[20]
8	MIDV	*Aedes spp.* (*A. leneatopennis, A. albothorax*)	Human, Horse	Africa	Ataxia, paralysis	Van Niekerk *et al.* (2015)[28]
9	MUCV	*Culex portesi*	Human	South America (including Brazil, Trinidad)	Encephalitis	Auguste *et al.* (2009)[25]
10	ONNV	*Anopheles* (*An. Gambiae* or *An. funestus*)	Human	Africa (including Gulu in Uganda, Kenya, Tanzania, Malawi, Mozambiques)	Lymphadenopathy-symmetricalpolyarthalgia	Levinson *et al.* (1990)[19]
11	RRV	*Ochlerotatus* spp. *V* (*O. igilax, O. camptorhynchus*, and *Culex annulirostris*)	Human, Horse	Australia (New South Wales, Ross River Valley in Queensland), Western Pacific, Solomon Islands, Indonesia	Polyarthritis, arthralgia, arthritis, rash	Atkins (2013),[32] Harley *et al.* (2001)[17]
12	SFV	*Aedes aegypti*	Human	Central, Eastern and Southern Africa (including Uganda)	Neurological disease (viral meningo-encephalo-myelitis)	Smithburn *et al.* (1946),[24] Mathiot *et al.* (1990)[21]
13	SINV	*Culex univittatus*	Human	Africa, Egypt, Israel, Philippines, Australia, Finland	Rash	Ehrengruber *et al.* (1999),[29] Jost *et al.* (2010)[30]
14	VEEV	*Culex*	Human, Horse	Yaracuy State (Venezuela), Northern countries of South America and Southern USA (including Florida), Messico	Encephalitis	Atkins (2013)[32]
15	WEEV	*Culex tarsalis*	Human, Horse	South America, USA	Encephalitis	Forrester *et al.* (2008),[15] Hahn *et al.* (1988),[16] Netolitzky *et al.* (2000),[23] Forrester *et al.* (2012)[31]

BFV, Barmah Forestvirus; CHIKV, Chikungunya virus; EEEV, Eastern Equine Encephalitis virus; EVEV, Everglades virus; GETV, Getah virus; HJV, Highlands J virus; MAYV, Mayaro virus; MIDV, Middelburg virus; MUCV, Mucambo virus; ONNV, O'nyong- nyong virus; RRV, Ross River virus; SFV, Semliki Forest virus; SINV, Sindibis virus; VEEV, Venezuelan Equine Encephalitis virus; WEEV, Western Equine Encephalitis virus. *in addition to aspecific febrile illness.

receptor binding. The receptor binding site is located at the A domain. The B domain, located at the distal end of the viral spike, prevents the premature activation of the E1 fusion-loop; in fact, the latter is located between A and B domains of E2.[34,39]

E3 is a 64 residues peptide with a key role in the maturation of the viral particles, during which it interacts with E2 and stabilizes the E1-E2 complex, ultimately preventing the premature activation of the fusion process.[41]

Alphavirus 6K is a 58-61 residues hydrophobic and acylated peptide, functioning as a viroporin. It is an integral membrane protein able to form ion channels across the plasma membrane, increasing its permeability and facilitating viral budding.[42,43] Another protein called transframe protein (TF) originates from the same coding region of 6K, through a frameshifting event that takes place at the C-terminal of the 6K sequence. TF presents regions rich in basic amino acids in close proximity of its transmembrane domain: a typical feature of viroporins.[42]

Viral replication

CHIKV is characterized by a broad tropism and it is able to infect and replicate within several vertebrate and invertebrate hosts.[10]

CHIKV life cycle starts with the attachment of viral particles on the surface of the target cells. In this context, E2 protein plays a key role in receptor binding. Although a cellular receptor has not yet been identified, glycosaminoglycans have been reported to increase viral adhesion and infection.[10,44-46]

Once viral particles have been recruited on the cell surface, the virus enters into the cell mostly through clathrin-mediated endocytosis.[47]

After its internalization, endosomal acidification causes the E1 protein to undergo a conformational change, exposing the fusion loop. The insertion of the latter in the endosomal membrane triggers the fusion of this host membrane with the viral envelope and the release of the viral particle into the cytosol.[10,46]

Subsequently, the disassembly of the nucleocapsid takes place and the viral genome is released. Once released into the cytoplasm, the positive-sense single-stranded RNA is translated into a long precursor for non-structural proteins called P1234. The latter is then cleaved into the P123 and nsP4 proteins that compose an early viral replicase required for the production of a negative-sense viral RNA.[48,49]

The synthesis of the negative-sense RNA occurs in special vesicular compartments, named spherules, localized at the plasma membrane. The dsRNA intermediates localize into the spherules that ensure them protection from degradation.[48]

Later during the infection, the complete proteolytic processing of ns-proteins results in a late-form replicase required for the synthesis of the new positive-sense RNA and the subgenomic RNA.[10,48,50] The latter encodes for a large polyprotein precursor of the structural proteins. Once translated, the capsid proteins are released from the polyprotein precursor through an autoproteolytic event and interact with the newly synthesized viral genome leading to its enclosure in the nucleocapsid.[51]

The translation of the other structural polyproteins leads to the production of two polyproteins: E3-E2-6K-E1 and E3-E2-TF. E3-E2-TF is a minor product that originates through a ribosomal frameshifting event occurring during 6K translation.[43,52]

In the host secretory pathway, the two polyproteins undergo cleavage and other post-translational modifications that lead to the production of the mature structural proteins. The mature E1/E2 proteins localize at the plasma membrane, where the acquisition of the viral envelope and the budding of the viral particles occur.[10,39]

Clinical features and pathogenesis in humans

Clinical manifestations

Based on clinical manifestations, Alphaviruses have been classified in two subgroups: encephalitogenic and arthritogenic.[53] CHIKV belongs to the latter subgroup including viruses that mainly cause rashes and polyarthralgia.

CHIKV is transmitted to humans through the bite of infected mosquitoes. Once into the human body, the virus spreads and replicates into skin fibroblasts and causes viremia. CHIKV is able to spread to lymphoid tissue, muscle, liver, joints and brain.[32] An incubation period of 3-7 days precedes the appearance of the signs of CHIKV-related illness.[54] The disease is characterized by a sudden onset followed by the increase of viral titers and activation of host innate immune responses.[10,55,56]

CHIKV symptoms comprise high fever, headache, chills, nausea, vomiting, myalgia, severe joint pain and swelling, maculopapular rash and, in some cases, diffuse lymphadenopathy and conjunctivitis, as represented in Figure 3. Although fever generally disappears in 3-4 days, it can

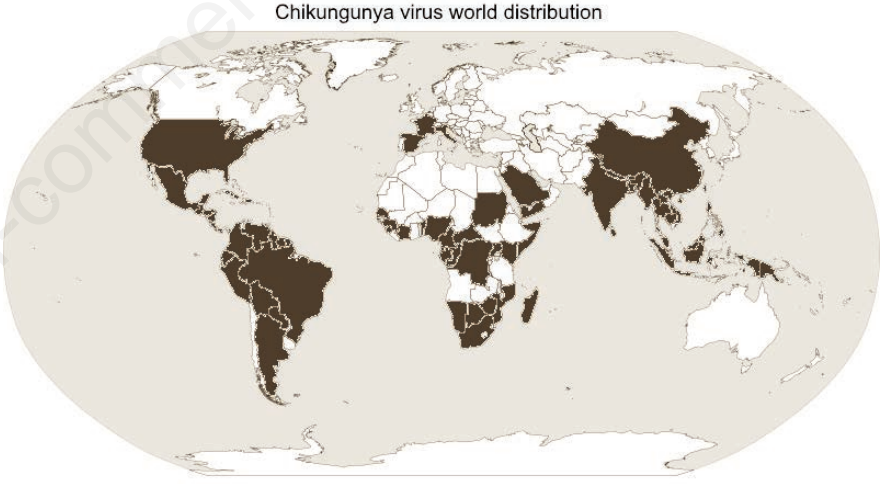

Figure 1. Map of Chikungunya virus (CHIKV) distribution in the world. Countries in which CHIKV outbreaks have been reported are depicted in grey.

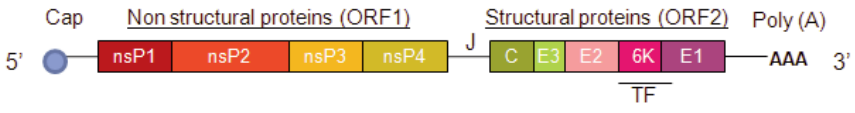

Figure 2. Chikungunya virus positive-sense single-stranded RNA genome [(+)ssRNA] with 5' cap, two central open reading frames (ORF1 and ORF2), linked by an untranslated junction region (J), and 3' polyadenylated tail. ORF1 and ORF2 respectively encode for non-structural and structural proteins.

occasionally exhibit a biphasic pattern with a second febrile episode after a fever-free period.[54]

In most cases, CHIKV disease is self-limiting and patients recover within 1-2 weeks.[57]

Importantly, about 30-40% of infected patients experience severe polyarthralgia for months or years.[27,58]

The joints affected by CHIKV polyarthropathy include knee, shoulder, hand and wrist.[54]

Joint swelling and tenosynovitis are common manifestations of CHIKV disease and pain can be so intense that common daily activities can be impared.

Other clinical manifestations attributed to CHIKV include neurological disorders such as encephalitis, myelopathy, myelitis and neuro-ocular disease, along with myocarditis and fulminant hepatitis.[54]

Moreover, CHIKV infection in the first trimester of gestation increases the risk of abortion.[54] Vertical transmission has also been reported.[59]

Chikungunya virus trasmission

CHIKV is an arbovirus native from Africa, widespread among primates through a non-human enzootic cycle sustained by arboreal mosquitoes mainly belonging to *Aedes* spp. Several species of mosquitoes have been identified as responsible for the dissemination of CHIKV in the enzootic cycle in Senegal, Ivory Coast, Central African Republic, Uganda and South Africa.[60-63]

In Senegal, CHIKV has been found in yellow fever virus vectors such as *Aedes (Diceromyia) furcifer*, *A.* (*Diceromyia*) *taylori*, *A.* (*Stegomyia*) *africanus*, *A.* (*Stegomyia*) *neoafricanus* and *A.* (*Stegomyia*) *luteocephalus.*[63] Occasionally, the enzootic cycle vectors can infect human hosts and contribute to inter-human transmission, giving rise to small outbreaks. When the virus reaches urban environments, the viral transmission cycle is supported by the anthropophilic vectors *A. aegypti* and *A. albopictus.*[64] These two species are able to generate endemic/epidemic outbreaks thanks to their characteristic of living in strong relationship with humans. In particular, *A. aegypti* females are strongly anthropophagous, preferring human blood for nutrition and often residing, for the entire gonotrophic cycle, in the same houses of the hosts they feed on.[65]

A. albopictus is a particularly aggressive vector, capable of transmitting up to twenty-six different arboviral infections. It is both zoophilous and anthropophile, active during day and night, able to produce diapausing, cold-resistant eggs.[66] In recent years, its reach is constantly expanding, to the point that it is considered responsible for the first outbreak recorded in Europe (Italy - Emilia Romagna Region), in 2007.[67]

CHIKV transmission is mainly horizontal and it occurs through the saliva of infected mosquitoes that is injected during a blood meal. A vertical transmission amongst the vectors, based on infected eggs, is also possible, although at lower rates.[68]

The viral cycle is usually divided into an extrinsic phase, when the virus is transported by the vector, and an intrinsic phase, when the virus is incubated in a vertebrate. The extrinsic phase begins when an uninfected mosquito swallows the virus during a blood meal from an infected vertebrate. The virus infects the mosquito's midgut and spreads through its body cavities, until it reaches the salivary glands. Thus, it is injected via saliva into a new host, giving rise to the intrinsic phase, in which it replicates inside the vertebrate.[7]

Epidemiology of Chikungunya virus disease

CHIKV was originarily based in Africa, where it is maintained in an enzootic cycle between forest-dwelling mosquitoes and non-human primates.[60]

Currently, the virus is endemic in Sub-Saharan India, Southeast Asia and Africa where, since its first isolation in 1952, the virus has periodically caused epidemics.[35,69] The frequency of viral epidemics is not predictable and outbreaks cyclically occur after an average 7-20 years interval from the last episode.[70,71] Nowadays, four different Chikungunya viral genotypes are known. They have been phylogenetically subdivided by nucleic acid sequencing techniques and named according to their geographic distribution at the time of isolation. East/Central/South African lineage (ECSA) is the genotype isolated in 1952, in East Africa.[72] A second viral genotype is represented by the monophyletic group called Asian lineage,[72,73] responsible for the first Asian outbreak, recorded in 1958, in Thailand. Since then, the virus has become endemic in that area, maintained in an urban cycle, mainly by *A. aegypti* mosquitoes.[33]

At the end of the twentieth century, a third viral lineage was isolated in Senegal and it was called West African (WA).[72,73]

In recent times (2004), an important CHIKV outbreak has emerged in Kenya, spreading to the Comoros and La Réunion, between 2005-2006, and involving other islands of the Indian Ocean, with more than 6 million probable cases.[74,75] It has been demonstrated that the strain responsible for this epidemic evolved from the ECSA clade; thus, it was called IOL.[76] During the outbreak in La Réunion, 90% of the isolates showed a non-synonymus mutation consisting of a single change, from alanine (A) to valine (V), at E1 envelope glycoprotein aminoacid 226 of the IOL viral genotype. This variant, called E1-226V, has an

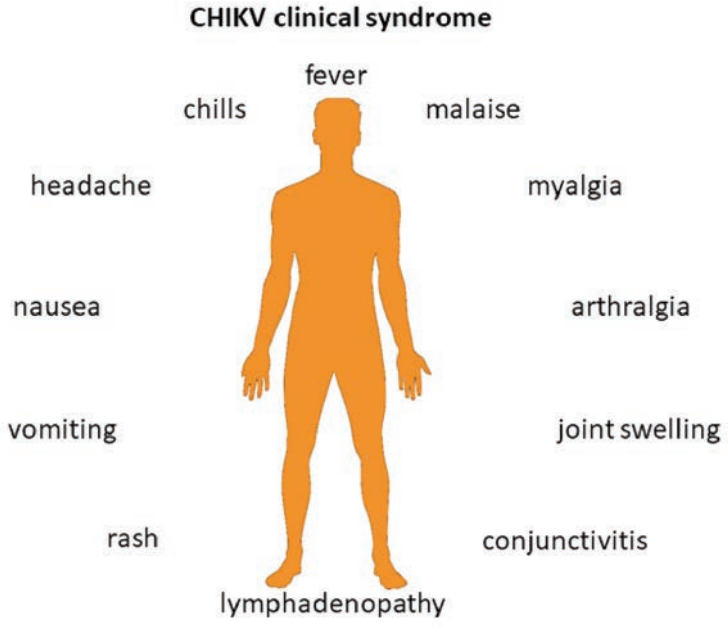

Figure 3. Chikungunya disease: major clinical manifestations.

approximately 40-fold larger efficiency to infect the midgut of the mosquito *A. albopictus*, a widespread vector in the country.[77] In 2005, this clade (IOL) and its variant (E1-226V) spread to India and, in the following ten years, reached all five continents, including, for the first time, Europe with an outbreak in Italy, in 2007.[67,78] In 2009, a second large epidemic began in La Réunion, spreading the virus all over the world, up to Europe, in Southeastern France, where CHIKVdisease was registered in September 2010.[79,80] In 2011-2012, CHIKV was reported in Central and Western Africa, Southern/Southeastern and Western Asia, Europe, Western Indian Ocean Islands and Oceania.[81]

An important outbreak started in the Caribbean Island of Saint Martin in December 2013 and, surprisingly, it was caused by the Asian genotype.[82,83] In February, the virus has expanded into French Guiana;[84] El Salvador was reached in June;[85] in July, the virus reached Florida, Costa Rica, Panama and Venezuela.[85] Subsequently, the virus was found in Brazil, Guatemala, Colombia, Nicaragua and Paraguay.[85] By November 2013, autochthonous transmission cases were recorded in Mexico, Belize and Honduras.[85]

In March 2015, the Pan American Health Organization declared 1,280,953 suspected cases of autochthonous transmission since the start of the epidemic, of which 26,200 confirmed.[85] In the same year, CHIKV disease was declared a notifiable condition by the Centers for Disease Control and Preventions.[86]

In 2017, two autochthonous epidemic events interested Europe and, in particular, Italy[87] and France.[88] At the time, 489 cases of CHIKV were reported in Italy, of which 270 were laboratory-confirmed. About 6% of infected subjects was hospitalized.

The same year, 125 imported cases of CHIKV occurred in Italy.[89]

The most recent outbreak was recorded in Sudan, from May to October 2018, with 13978 cases, none requiring hospitalization.[90]

The diffusion of CHIKV is strongly linked to the diffusion of its vectors. The contrast of mosquito breeding through disinfestation activities, combined with empowered risk awareness among the population living in areas with high density of vectors, are probably the most effective strategies to fight the expansion of the virus. The adoption of methods for the prevention of mosquito bites (*e.g.* the use of repellents, wearing long-sleeved clothing, resting in screened or air-conditioned rooms and using mosquito bed nets) should be encouraged. Above all, surveillance and coordination by health authorities need to be organized and described in precise directives. An example is represented by the Italian case, where in response to the outbreak recorded in 2017, the health authority issued the so-called Plan for surveillance and response to arbovirosis transmitted by mosquitoes (*Aedes* spp.) with particular reference to Chikungunya, Dengue and Zika viruses.[91] The aims of this national program are: monitoring of the cases imported in Italy, particularly in potentially vector-dense areas; early identification of epidemics and active monitoring of local transmission (diffusion, entity and term), in order to adapt Public Health measures (prevention and response activities) and to direct interdisciplinary control activities of the carrier; prevention of accidental transmission of CHIKV that can also occur iatrogenically through infected blood and blood components or cells, tissues, organs; identification of further potential transmission modalities (*e.g.* sexual contacts).

Laboratory diagnostics, therapy and vaccines

Chikungunya virus diagnosis

CHIKV and Dengue virus can be both transmitted by *A. aegypti*; reportedly, these viruses can co-infect the same mosquito and be transmitted together.[92] This event leads to CHIKV/Dengue twin outbreaks. As these viruses present some significant similarities in clinical manifestations, aetiological diagnosis through clinical observation can be difficult in the areas of the world where both Chikungunya and Dengue viruses are endemic. Due to this, laboratory confirmation is required to reach a proper diagnosis.

Polymerase chain reaction (PCR), quantitative reverse transcription PCR (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA) are the most common techniques used for this purpose.

CHIKV reaches high titer within a week after infection.[93] This allows early diagnosis of CHIKV infection through the detection of the viral genome in the serum of infected subjects 7 days after symptoms onset.[93]

The available PCR and RT-qPCR assays permit the detection of structural and non-structural CHIKV genes.[2] Moreover, RT-PCR can be used in combination with DNA-sequencing for viral genotyping.[2]

In addition to molecular techniques, serological tests (*e.g.* ELISA) can be applied for the detection of IgM and IgG antibodies against CHIKV. IgM appear few days post-infection and can be detected for months;[94,95] instead, IgG can be found months to years after the onset of the disease.[96] The major limitation of serological methods for CHIKV identification is represented by the cross-reactivity versus dengue and other arboviruses that can lead to false positive results.[74,97]

Therapy

Nowadays, no specific treatment is available against CHIKV; therefore, current therapies only allow symptoms relief and general support.

In 2016, Brito *et al*. proposed new guidelines for the pharmacological treatment of CHIKV-related acute and chronic joint pain.[98] These guidelines highlight treatment differentiations based on pain evaluation, suggesting the use of the Visual Analog Scale for pain level assessment. The authors provide different protocols for the treatment of mild, moderate and intense acute and chronic pain.

The use of analgesics, such as paracetamol or dipyrone, is suggested in case of mild pain; their combination is recommended in case of moderate pain. For the treatment of intense pain, the aforementioned analgesics can be combined with opioids, like codeine or tramadol. In front of a neuropathic pain diagnosis, amitriptyline or gabapentin are indicated. Patients that experience moderate or severe pain in sub-acute phase can be treated with corticosteroids. In subjects with chronic pain, hydroxychloroquine, sulphasalazine or methotrexate are considered the treatment of choice.

Chikungunya virus vaccines

At the moment, no effective CHIKV vaccine is commercially available; however, some promising vaccines are undergoing clinical trials.

An interesting candidate is represented by the MV-CHIKV vaccine. This consist of a measles virus vector harboring an additional transcriptional unit between the P and M genes.[99] This transcriptional unit comprises the cDNA of the CHIKV structural proteins C, E3, E2, 6K and E1. Once translated, these proteins are able to assemble and give rise to CHIKV viral-like-particles that are secreted by infected cells. CHIKV sequence used for the MV-CHIKV vaccine comes from the La Réunion strain 06-49.

The safety and the immunogenicity of this vaccine have been evaluated in a phase I clinical trial in 2015[100] (EudraCT, number 2013-001084-23). The MV-CHIKV vaccine showed a good immunogenicity with 100% seroconversion in all the subjects after two doses of the vaccine and a good safety profile with no serious vaccine-related side-effects reported. The safety and immunogenicity of this vaccine have been further

evaluated in a phase II clinical trial completed in April 2018 (NCT number: NCT02861586). An additional phase II clinical trial, designed to compare tolerability and long-term immunogenicity of different vaccine formulations is now ongoing (ClinicalTrials.gov Identifier: NCT03635086).

Another promising candidate is the viral-like-particle (VLP) based vaccine VRC-CHKVLP059-00-VP. The VLP consist of capsid, E1 and E2 proteins. This vaccine is based on the West African strain 37997, but it is thought to induce cross-protection against different viral strains. Safety and immunogenicity of this vaccine have been evaluated in a phase 1 clinical trial involving 25 participants aged between 18-50 years (ClinicalTrials.gov, NCT01489358). The results of this clinical trial revealed that the vaccine is well tolerated, as no severe side effects have been reported, and immunogenic with neutralizing antibodies detected several months after the vaccination.[101] A phase 2 clinical trial involving 400 participants from CHIKV endemic areas is currently ongoing (NCT number: NCT02562482).

In addition to the viral-like-particles-based vaccines, a live-attenuated CHIKV vaccine candidate is now in a phase 1 clinical trial (NCT number: NCT03382964).

The live-attenuated virus vaccine (VLA1553), produced by the Valneva biotech company, has been obtained through the deletion of a part of the nsP3 gene.

In pre-clinical trials a single shot of VLA1553 vaccine was sufficient to induce the production of a high-titer neutralizing antibodies and a good T-cells response in C57BL/6 mice. The induced immune response protected the mice against viremia and joint swelling.[102]

Furthermore, 120 healthy subjected aged between 18 and 45 years have been recruited in the ongoing clinical trial. The aim of this study is to compare the immune response and the eventual side-effects induced in three groups of enrolled subjects, respectively treated with low, medium or high doses of vaccine. The results of this clinical trial are expected for June 2019.

Conclusions

Due to its high morbidity and diffusion, CHIKV infection is now considered an important global public health concern.

Since 1952, year of its first isolation, the virus has caused sporadic and epidemic outbreaks with an unpredictable re-emergence pattern.

Currently, CHIKV infection has been reported in almost 40 countries.[103]

Different factors have probably contributed to CHIKV worldwide expansion, including the wide distribution of its vectors, their transmission efficiency and the travel of infected subjects to areas of the world in which competent mosquitos are available.[103,104]

A noteworthy example is represented by the first European outbreak reported in Italy, in 2007. This outbreak, involving 217 subjects, was caused by an Indian subject who became symptomatic two days after his arrival in Italy.[105]

Nowadays, the re-emerging and the spread of CHIKV in countries different from those of origin have caused several millions of cases worldwide, increasing the interest in the study of this arthropod-borne virus. This has led to improved protocols for the symptomatic treatment of CHIKVdisease and to the development of different promising vaccines that are undergoing evaluation in clinical trials.

Despite a risen attention, several aspects of CHIKV infection, such as virulence factors and pathogenetic mechanisms, are still poorly understood and no specific antivirals are available.

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