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Research on reemerging of chikungunya - virus disease

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ABSTRACT

The virus causing Chikungunya disease was identified over 60 years ago. Research interest in the disease increased after an important epidemiological outbreak occurred in 2005 on the French metropolitan island of La Reunion located in the south-eastern part of the Indian Ocean. In 2007, a smaller outbreak of Chikungunya developed in the north-eastern part of Italy made possible by immigration of a viremic patient from the Indian Ocean area and the enormous population of Aedes albopictus in Italy. Currently, Chikungunya is spreading in Southeast Asian aspects, clinical pictures, diagnosis and treatment of the disease caused by Chikungunya virus.

Keywords: Chikungunya, *Aedes albopictus*, arbovirus.

INTRODUCTION

History and epidemiology of Chikungunya virus infections in the world

Human infections caused by Chikungunya virus (CHIKV) were reported for the first time in East Africa some five decades ago in 1952-53 during an epidemic of fever that developed along the border between Tanzania and Mozambique. This fever was again later described by M. Robinson and W.H.R. Lumsden in 1955 and the disease was reported be very similar to a "Dengue-like fever". The name"Chikungunya" was derived from a word in the Makonde

language (spoken by a population that lives in the Mozambique region) that means "that which bends up". This description refers to the stooped posture that develops as a result of the arthritic symptoms of this disease. Since the first report in 1955, most cases of CHIKV infection have been described in Africa and India; however, during the last 60 years, isolated cases or epidemic outbreaks of CHIKV infection have been reported in India, and in several countries in Africa, the Indian Ocean region, and Southeast Asia. In detail, CHIKV has been reported in the following African countries: Benin, Burundi, Cameroon, Central African Republic, Kenya, Uganda, Malawi, Senegal, Congo, Nigeria, Sudan, Guinea, South Africa, Tanzania, Zimbabwe, Namibia, Comoros, Mayotte, Ghana, Burkina Faso, Mozambique, and Gabon. Most of the CHIKV cases in Asia were reported in India, Sri Lanka, Myanmar, Thailand, Vietnam, Taiwan, Singapore, Cambodia, Pakistan, Laos, Philippines, Malaysia, Indonesia, and East Timor. In the Indian Ocean area CHIKV infections have been recently reported from Seychelles, Madagascar, Mauritius and La Reunion. Isolated or small clusters of imported cases have also been found in European or North American countries (Canada and USA). The only epidemic of CHIKV that arose outside tropical areas was the small epidemic in the Emilia Romagna region in Italy during the summer of 2007. Among the largest epidemic outbreaks of CHIKV infection is the one reported from India and Sri Lanka in 1962, which infected more than 100,000 persons and caused 200 deaths. While the cases are yet to be enumerated, several million persons are thought to be involved in the CHIKV outbreaks of the first half of this decade [4, 5].

More recently, the CHIKV diffusion area moved westbound, involving many islands of the Indian Ocean .In particular, between February 2005 and March 2006, CHIKV infection involved more than 244.000 inhabitants of the French island of LaReunion, located in the Indian Ocean, off the eastcoast of Madagascar. During this epidemic episode an estimated death toll of 203 persons was associatedwith CHIKV. From January 2006 until August 2007 more than 1,000,000 cases of CHIKV-related disease have been estimated in different parts of India. The highest incidence rates were reported from the bordering southern Indian Federal states of Kerala and Tamil Nadu. In the same period, smaller epidemics (involving some 15,000 people) were reported in Indonesia and in the western part of Africa (Gabon) during January to August 2007.

Viral structure, genome organization and transmission cycle of CHIKV

CHIKV is an arthropod-borne virus (arbovirus) belonging to the *Alphavirus* genus of the *Togaviridae* family [12]. Electron microscopy study of CHIKV showed a characteristic alphavirus morphology: a size of 50-70 nm, an icosahedral-like nucleocapsid surrounded by an envelope with embedded viral glycoproteins .The nucleocapsid contains the single-stranded plus-sense RNA genome of approximately 11.8 kb complexed with multiple copies of a single species of capsid protein (C) of about 30 kDa. The virion envelope consists of a lipid bilayer, derived from the plasma membrane of the host cell, in which are embedded multiple copies of two major virus encoded glycoproteins El and E2 [15] as well as another small peptide, 6K, that is associated with virus particles only at a very low level .E1 and E2 proteins both have a molecular mass of about 50 kDa and are anchored in the membrane by conventional membrane-spanning anchors located in their C-terminal regions. These two polypeptides form a stable heterodimer and threeE2-El heterodimers interact to form the spike that is found on the virus surface. The encapsidated genome consists of a non-segmented, single-stranded, positive-sense RNA with a 5'- terminal methylguanylate cap and a 3' polyadenylation. CHIKV genome transcription and replication are entirely cytoplasmatic and the virus enters the target cells by

endocytosis of clathrin-coated vesicles. The assembled virus particles finally bud through the cell membrane and become enveloped virions . CHIKV infection is enzootic across tropical regions of Africa and Asia and the virus is transmitted by mosquitoes belonging to the species of the *Aedes* genus: *A. aegyptii* and *A. albopictus*. Mosquitoes such as *A. albopictus* are abundant and widely distributed in urban areas of Europe and the (USA) States of America, and they have the potential to expand the enzootic area of the virus. *A. albopictus* was involved in CHIKV transmission between humans in La Reunion Island and, more recently, in Italy. Phylogenetic analysis based on the *E1* gene or on the complete genome sequences of strains isolated in Africa, Asia and during outbreaks in 2005-2007 in the islands of the Indian Ocean and in India revealed the existence of three distinct CHIKV phylogenetic groups: one containing all isolates from West Africa; one containing isolates from Asia; and one corresponding to Eastern, Central and Southern African (ECSA) isolates .It is interesting to note that the 2005-2007 epidemics registered the introduction of ECSA genotype in Asia. The strain that caused the first outbreak in Europe (Italy, 2007) belongs to the ECSA group.The viral isolates obtained during this last European outbreak, clustered with strains identified during the recent outbreak in the Indian Ocean islands, contained a genetic change in the position 226 of the gene for the membrane fusion glycoprotein E1 (E1-A226V) .This point mutation was shown to be responsible for an increased capability of these CHIKV strains to infect and replicate in *A. albopictus*, facilitating virus transmission to a naïve human population .

Clinical features, pathogenesis and host immune response

Chikungunya fever is an acute illness characterized by a sudden onset of high fever, rash and joint pain. The most significant symptom of CHIKV-related disease consists of a painful arthralgia that occurs in almost 100% of patients. Most infections completely resolve within weeks but there are reported cases of CHIKV-induced arthralgia lasting for months, or even for years, in the form of recurrent or persistent episodes. The pathogenesis of Chikungunya disease remains unknown, contrasting with related alphaviruses such as Sindbis virus (SINV), Semliki Forest virus (SFV), and Ross River virus (RRV). This lack of information probably reflects the fact that, up until recently, CHIKV infection has spread mostly in developing countries. Former studies (1960s-1980s) demonstrated that CHIKV replicates in various non-human cell lines, including Vero cells, chick embryo fibroblast-like cells, BHK21, L929 and Hep-2 inducing significant cytopathic effect (CPE) .Recently, Sourisseau *et al.* reported the interaction of CHIKV with human cell lines, demonstrating that various human adherent cells, including epithelial and endothelial cells, primary fibroblasts as well macrophages were susceptible to CHIKV infection. In contrast, T and B lymphocytes and monocyte-derived dendritic cells (DCs) did not allow viral replication. In addition, immunohistological studies on muscle biopsies from two CHIKV-infected patients with myositic syndrome showed the presence of viral antigens, located exclusively inside skeletal muscle progenitor cells (designed as satellite cells) and not in muscle fibers. Since these cells are considered the main cell type responsible for postnatal muscle growth and repair, the susceptibility of satellite cells to CHIKV infection is possibly due to the persistence of the virus on muscle tissue leading to the recurrent myalgia, often experienced by some patients . Moreover, it was shown in a mouse model, as well as in humans, that the fibroblast is the major cell type targeted in *vivo* by CHIKV. This observation confirms previous *in vitro* findings,and accounts for CHIKV tropism for muscles, joint and skin connective tissues .

The host immune response to CHIKV infection remains unexplored. Recently, a mouse model for Chikungunya infection was developed, suggesting that CHIKV-induced arthritis is of immunopathological origin and that the severity of CHIKV infection is critically dependent on two host factors: age and functionality of the type-I Interferon (INF) signaling system .Whereas it is clear that an increased neonatal susceptibility is also observed in humans , the relevance of a role for type-I INF in severe CHIKV infection in adult humans remains to be demonstrated.

Furthermore, Ng*et al*. reported a predominance of type 2 circulating cytokines, mainly IL-5, IL-6 and IL-10, in acutely CHIKV-infected patients, hypothesizing an anti-inflammatory response late during infection (when the virus is being eliminated from blood) that might counter-balance the inflammatory response occurring earlier when the virus is actively replicating. The interpretation of the significance of these studies at the moment is only speculative.

Diagnosis

The laboratory diagnosis of CHIKV infection is routinely achievable by different methods, including the isolation of the virus in cell culture from plasma or serum; the detection of the viral RNA by RT-PCR in serum; and the evaluation of the CHIKV-specific serologic antibody response (IgM and/or IgG antibody detection or identification of an antibody mediated viral neutralizing activity in serum). Virus isolation is based on inoculation of the biological sample in cell cultures, derived either from mosquitoes or from mammals. As an alternative, the specimen for CHIKV detection can be inoculated into suckling mice. This last method is, however, labour intensive and time-consuming. To detect the presence of viral RNA, several real-time RT-PCR protocols, targeting the nsp1 or *E1* gene, have been developed. Molecular tests are capable of detecting the viral RNA only during the viremic phase in patients, which usually lasts from day 0 to day 6 after the clinical onset [Parola and colleagues , analysed serum samples from four infected travellers returning from the Indian Ocean islands by using a quantitative real-time RT-PCR-based method and detected a viral load up to 10^9copies/ml in one case these findings suggested that the veremia can reach very high concentration during the symptomatic phase of CHIKV infection. Such high levels of viremia are uncommon in other arthropod-borne diseases such as dengue fever and West Nile disease. No clear information is presently available about the viral load that is present in serum during the pre-symptomatic stage of infection, which likely lasts for some days before the clinical onset. CHIKV specific IgM and IgG antibodies are detectable in plasma and serum samples from acutely infected and convalescent patients by the following classic serological methods: inhibition of the haemagglutination, complement fixation, immunofluorescence (IIF) and immunoenzymatic assays (ELISA). The IgM specific response against CHIKV is detectable starting from two to six days after the onset of symptoms by ELISA and IIF, and could persist for several weeks up to three months. The IgG antibodies are present in sera from convalescent stage patients and usually persist for several years. All the above reported serological methods are highly sensitive but only moderately specific: this phenomenon is mainly due to the antigenic cross-reactivity between CHIKV and other arboviruses such as Dengue virus, o'nyong-nyongvirus, Sindbis virus, and many others.

Furthermore, as the clinical symptoms of CHIKV infection resemble those of several other febrile arthropod-borne infections (mainly Dengue), a differential laboratory diagnosis based on

confirmation methods is required either in patients living in endemic countries or in travellers returning from tropical areas that were detected as IgM or IgG positive by standard serological tests. Confirmation is generally achieved by performing a plaque neutralization test (PRNT) *in vitro*: this assay is time-consuming and labor intensive and can be routinely performed by third level laboratories that act as reference structures. Up to now, in-house ELISA and IIF tests were mainly used for CHIKV diagnosis. Recently, some commercially available serologic assays, including IIF and ELISA, were developed and their sensitivity and specificity values were evaluated and assessed.

Treatment and vaccination

Currently, no specific antiviral treatment for CHIKV is available. Therapy is therefore purely based on the patient's symptoms. Analgesics, antipyretics, and anti-inflammatory agents are the most appropriate treatments for the primary signs, which include fever and joint pain with swelling .For chronic cases of arthritis subsequent to CHIKVinfection, chloroquine phosphate was demonstrated to provide relief to patients with limited response to non-steroidal anti-inflammatory drugs. In addition to this drug therapy, actions including bedrest and fluids are recommended.

The recent outbreaks of CHIKV in Asia and in Italy stimulated renewed interest in developing new antiviral agents. RNAi, for example, is emerging as a new strategy to fight virus infections. There is currently no commercial vaccine for Chikungunya virus, although some candidate vaccines have been tested in humans. For a complete review of the vaccine studies performed in the past 40 years against CHIKV, Levit *et al.* Harrison *et al.* reported the development of a very promising cell culture based formalin inactivated vaccine. The high levels of neutralizing antibodies in human volunteers in a phase 1 trial, and the excellent immunogenic response without showing any adverse effects made it a good candidate for future use. Edelman *et al.* developed an attenuated vaccine by serial passages of CHIKV in an MRC-5 cell line; this vaccine preparation was highly immunogenic and well tolerated. In the phase II safety trial, satisfactory sero-conversion rates (98% on day 28) were observed with neutralising antibody titres, persisting in 85% of cases when evaluated after one year from the last shot. After the 2005-2006 epidemics in the Ocean Indian area, several differentattempts have been made to revive the project by the US Army Medical Research Institute for Infectious Disease following a request from the French government.

The appearance of Chikungunya virus in Italy: an example of the spreading of a re-emerging disease in new climates

During the outbreak on the Indian Ocean islands,a large number of travellers from industrialised countries with temperate climates became infected with CHIKV. Upon returning to their native countries these travellers were still positive for the virus. In Italy, the national infectious diseases surveillance system reported 22 returning travelers with symptoms of CHIKV infection from the Indian Ocean islands (Mauritius, Reunion, Madagascar) between January 2006 and March 2007 who were CHIKV serum positive. Afterwards, local transmission of CHIKV was reported in Emilia-Romagna, Italy, during the summer of 2007. During this epidemic outbreak, 205 cases of CHIKV infection were identified between July 4 and September 27, 2007. The presumed index case was a man from India who developed fever and joint pain two days after his arrival in Italy, who was probably highly viremic when visiting his relatives in the village where the outbreak

began. CHIKV sequences were detected by PCR in human blood and from *Aedes albopictus* captured during the epidemic episode. Human and mosquito strains clustered with Indian CHIKV viral isolates and contained the same mutation (Ala226Val) in the glycoprotein E1 gene that had also been found in virus variants circulating in the Indian Ocean islands. This mutation has allowed the virus to have increased infectivity to the *A. albopictus* mosquito compared to other *Aedes* spp. Together the high density of *A. albopictus* in Italy at the time of the index case arrival and the high virus- vector fitness of CHIKV variant strain favoured the rapid spread of local transmission of CHIKV reported in Emilia-Romagna. This epidemic of CHIKV infection was finally caused by the concomitant presence of a large population of highly competent vectors and an individual returning from an CHIK epidemic area while in the asymptomatic highly viremic stage In fact, during the outbreak, the occurrence of new cases in the initially affected area started to decrease a few days after vector control measures were implemented. The measures for controlling the population of A. *albopictus* included the use of fast-acting insecticides (synergised pyrethrumcompounds) for three days consecutively and the implementation of anti-larval measures using formulations of insect growth regulators and *Bacillus thuringiensis* var. *israeliensis*. House-to-house interventions were done to eliminate breeding places, and community participation was encouraged. For each suspected case of infection, these control measures were implemented within a radius of 100 m of the affected individual's residence; for clusters, the control measures were done within a 300 m radius of the most external case.

Overall, the epidemic in Italy was the result of the combined effect of the globalisation of vectors and humans, which occurred through the introduction and adaptation of the vector *A. albopictus* into a temperate country and the introduction of CHIKV through an infected person in the viremic phase in a previously infection-free country with totally susceptible population

Since its introduction in Italy in 1990, *A. albopictus*, commonly named Asian tiger mosquito, spread quickly across the country. The source of *A.albopictus* infestation in Italy was identified as a warehouse of a tire rethreading company that had imported used tires infested with mosquitoes eggs from Georgia. In Italy, two major tire rethreading companies, located in the outskirts of Padova and Bologna (Veneto and Emilia-Romagna Regions respectively), that imported scrap tires directly from the USA, allowed the quick spread of the mosquito across the country throughout the internal trade of the tires sent to smaller companies. Unfortunately, despite efforts made to control the spread of *A. albopictus* mosquitoes, the insect became well established in almost the entire national territory. Two main factors allowed this species to quickly and strongly establish in Italy: 1) the geographical origin (USA) of the imported vector populations meant that the mosquitoes were able to survive to the cold season of the northern hemisphere because of egg diapauses; 2) the mosquito's notable biological plasticity to different environmental conditions such as kinds of breeding sites, housing, and different hosts suitable for the blood meal .Unlike other mosquitoes, the *A. albopictus* eggs can withstand desiccation, which allows them to travel around the world in a variety of containers. Atpresent, scattered foci of the tiger mosquito diffusion are reported in all the regions of Italy, with the exception of Valle d'Aosta, and in 82 out 107 provinces, from coastal plains to inner lands up to altitudes of 600 m. In the Emilia- Romagna region, most cities that are located in areas below 500 m a.s.l. are infested by *A. albopictus* during the April-October period.

CONCLUSION AND FUTURE DIRECTIONS

The Emilia-Romagna region has implemented a surveillance system against the Asian tiger mosquito infestation based mainly on the use of ovipositor traps and on the active search of adult mosquitoes and larvae. After the outbreak recorded in summer 2007, a regional surveillance system was set up and the 2008 Emilia-Romagna Regional Preparedness Plan for Chikungunya and dengue was approved and released by the Health Authority. The plan was launched because, considering the difficulties in controlling the spread of the vector mosquitoes and the large number of people travelling to and from endemic areas for CHIKV infections, the regional authority had thought that the 2007 epidemic could have been only the first of a possible series of outbreaks. The Regional plan has been launched for the prevention of both Chikungunya and dengue fever, even though *A. Albopictus* is competent for the transmission of a larger number of Arboviruses. This choice was based on epidemiological criteria, the similarity of both diseases from a clinical point of view, and the practicability of a single surveillance system. This plan increased the alert level of the whole public health system in the region and ruled the activities to control the diffusion of *A. albopictus.* The prevention of Chikungunya and dengue fever was based on early detection of human suspected cases and immediate implementation of environmental control measures against *A. albopictus*. One of the objectives of the plan was the assessment of the level of tiger mosquito infestation in all provinces and in major urban centres by increasing the number of ovitraps to get a quantitative definition of the presence of *A. albopictus*. Monitoring by means of ovitraps, based on the number of eggs laid in containers which attract pregnant female mosquitoes, is an indirect surveillance method which can provide information about the development of the adult population. Moreover, the plan established several control measures against the proliferation of the Asian tiger mosquito. Ordinary control measures are based on periodic larvicidal treatment in public road drains (manholes, basement window wells, etc.) as well as information from and the involvement of citizens regarding the management of their own gardens. Adulticide of mature mosquitoes has to be performed in sensitive sites such as schools, hospitals, etc. upon the client's request and after hearing the opinion of the local public health unit. A specific and targeted strategy in those areas where indigenous CHIKV cases occurred in 2007 envisages the extraordinary "door-to-door" pest control in private properties with larvicidal treatment of the breeding sites that cannot be eliminated and removal of all potential larval breeding sites that can be eliminated[9-14].

According to the 2008 Regional Preparedness Plan, a person diagnosed with a clinical criterion, such as sudden onset of fever greater than 38.5°C and invalidating arthralgia, fulfilling or not the epidemiological criterion (travelling to an endemic or epidemic area for this disease) is considered a suspected case of CHIKV fever and laboratory diagnosis must be performed. The outbreak of CHIKV infection that occurred in summer 2007 in a temperate area of northern Italy constitutes a new model for the diffusion of a tropical disease outside the conventional locations; this situation has been caused mainly by the dangerous mixture of the large population of a highly competent vector, the Asian tiger mosquito, and the possibility that an individual comes back from the area of normal diffusion of CHIKV during the asymptomatic viremic stage. Considering the difficulties in controlling the spread of A. *albopictus* and the large number of people travelling to and from the areas of normal diffusion of vector-borne tropical diseases, we think that the 2007 epidemic could be only the first of a possible series of these outbreaks. In conclusion, this urban epidemic of CHIKV infection in a temperate country determines a new

perspective in the preparedness to unexpected emerging virus infections that must be faced by using a combined strategy of vector diffusion monitoring and immediate diagnosis of any suspected cases of imported vector-borne exotic disease.

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