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Review

Chikungunya virus pathogenesis: From bedside to bench

Thérèse Couderc [a,b], Marc Lecuit [a,b,c,d,*]

[a] Institut Pasteur, Biology of Infection Unit, Paris, France
[b] Inserm U1117, Paris, France
[c] Paris Descartes University, Sorbonne Paris Cité, Division of Infectious Diseases and Tropical Medicine, Necker-Enfants Malades University Hospital, Institut Imagine, Paris, France
[d] Global Virus Network

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ABSTRACT

Chikungunya virus (CHIKV) is an arbovirus transmitted to humans by mosquito bite. A decade ago, the virus caused a major outbreak in the islands of the Indian Ocean, then reached India and Southeast Asia. More recently, CHIKV has emerged in the Americas, first reaching the Caribbean and now extending to Central, South and North America. It is therefore considered a major public health and economic threat. CHIKV causes febrile illness typically associated with debilitating joint pains. In rare cases, it may also cause central nervous system disease, notably in neonates. Joint symptoms may persist for months to years, and lead to arthritis. This review focuses on the spectrum of signs and symptoms associated with CHIKV infection in humans. It also illustrates how the analysis of clinical and biological data from human cohorts and the development of animal and cellular models of infection has helped to identify the tissue and cell tropisms of the virus and to decipher host responses in benign, severe or persistent disease. This article forms part of a symposium in *Antiviral Research* on "Chikungunya discovers the New World".

Contents

* Corresponding author at: Institut Pasteur, Biology of Infection Unit, Paris, France.
E-mail addresses: therese.couderc@pasteur.fr (T. Couderc), marc.lecuit@pasteur.fr (M. Lecuit).

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1. Introduction

Chikungunya virus (CHIKV) is a member of the *Alphavirus* genus, belonging to the *Togaviridae* family. Several alphaviruses cause disease in humans. They are divided in two main phylogenetically distinct groups: one that causes arthralgia and/or arthritis, mainly found in the Old World and which includes CHIKV and its closest relative Semliki Forest virus (SFV), O'Nyong Nyong, Ross River (RRV), Barmah Forest (BFV); and one that causes encephalitis, mostly found in the New World and that includes western equine encephalitis and Venezuelan equine encephalitis viruses (for review see Griffin, 2007). Sindbis virus (SINV), which is geographically restricted to the Old World, is however phylogenetically closer to the New World subgroup (Griffin, 2007). Upon mosquito bite, CHIKV induces an acute febrile illness typically accompanied by severe arthralgia, which can last and relapse for weeks to months. CHIKV has been the cause of several outbreaks in Africa, from where it originates and was first identified in the 50s, and in Asia. Since 2005, a new virus lineage (called Indian Ocean Lineage, IOL) that originated from Africa, has caused a massive outbreak in the Islands of the Indian Ocean, and reached India, South-East Asia, and also led to clusters of autochthonous cases in Southern Europe. Since the end of 2013, a strain of CHIKV originating from Asia has emerged the Caribbean and spread to South, Central and North America (Weaver and Forrester, 2015; Weaver and Lecuit, 2015).

Here we review the current knowledge on CHIKV infection mainly obtained from the analysis of cohorts of human patients and experimental animal models.

2. Clinical presentation of chikungunya in humans: more than a benign disease

CHIKV infects human through the bite of mosquito vectors and causes disease called chikungunya, which means "walking bent" in Makonde, a language spoken in Austral Africa, where it was first identified (for review see Burt et al., 2012; Morrison, 2014; Staples et al., 2009; Suhrbier et al., 2012; Thiberville et al., 2013b; Weaver and Lecuit, 2015) (Fig. 1). Its symptoms are similar to classical dengue fever, except that they are associated with intense arthralgia, which is strongly predictive of chikungunya. The incubation period is short, lasting about 2–4 days (Fig. 2). In contrast to dengue fever, asymptomatic infections are rare; roughly 3–25% of people with serological evidence of infection have no obvious symptoms. CHIKV infection is usually self-limited, non-fatal, with fever resolving within a few days. However, since the Indian Ocean outbreak in 2005–2006 (Schuffenecker et al., 2006), the information available about the clinical characteristics of the human disease has significantly increased with the detailed clinical study of cohorts of CHIKV-infected patients, notably in the Island of La Réunion, a French overseas department (Staikowsky et al., 2009). Previously unreported severe forms of CHIKV infection were observed, as well as maternal–fetal transmission (Economopoulou et al., 2009; Gerardin et al., 2008; Rajapakse et al., 2010). The most notable clinical feature of chikungunya is related to the fact that, following the acute phase, joint symptoms may persist for weeks to months and even years, with possibilities of relapses, leading to arthritis and some cases destructive rheumatism, with pathogenesis has yet to be fully understood (Queyriaux et al., 2008).

2.1. Chikungunya fever in humans

The incubation period ends with a sudden onset of high fever (>39 °C), back pain, myalgia, associated to severe and incapacitating arthralgia, together with headaches, photophobia, and rash (for a review see above) (Figs. 1 and 2). The onset of fever coincides with viremia, and blood viral load can rapidly reach up to 10^9 viral genome copies per milliliter (Parola et al., 2006; Staikowsky et al., 2009) (Fig. 2). Viral replication triggers the activation of innate immune responses, the hallmark of which is the production of type I interferons (IFNs) (Schwartz and Albert, 2010).

A positive correlation between the intensity of viremia and acute illness has been observed. Actually higher viral loads have been found in hospitalized patients with comorbidity than those without (Staikowsky et al., 2009) and is most often associated with

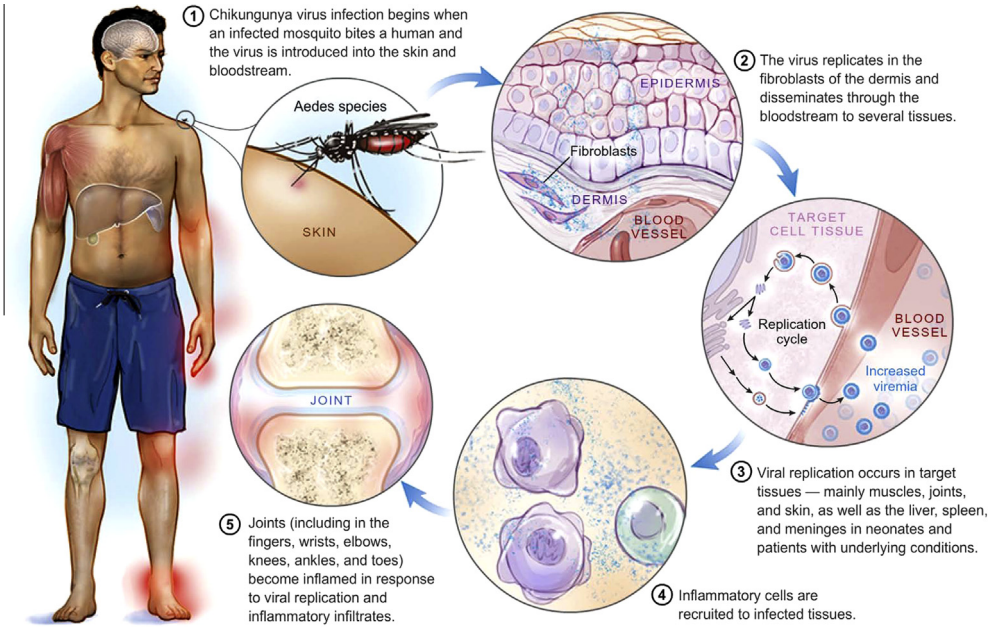

Fig. 1. Successive steps of chikungunya virus infection in humans, based on human clinical data and animal experiments. 1. CHIKV inoculation by mosquito bite. 2. Viral replication at the inoculation site, in dermal fibroblasts. 3. Replication in target tissues, with recruitment of inflammatory cells to infected tissues. 4. Joint inflammation. Adapted from Weaver and Lecuit (2015). Copyright Massachusetts Medical Society 2015. Reproduced with permission.

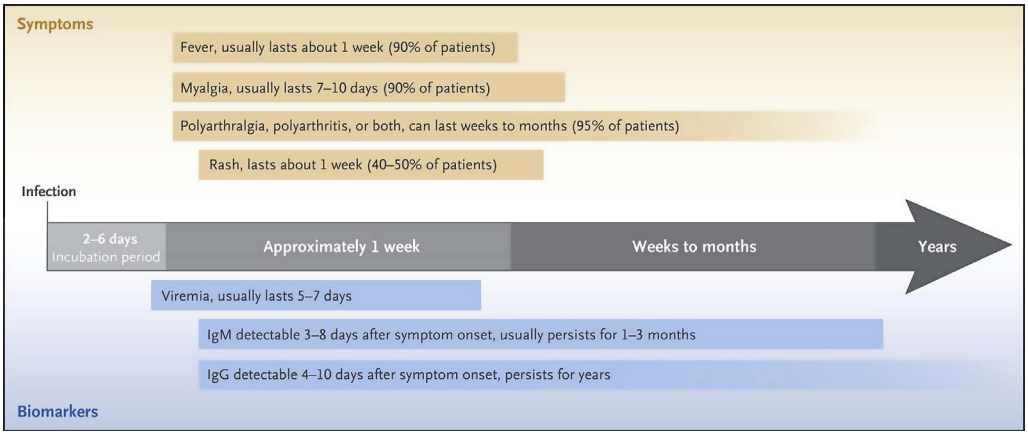

Fig. 2. Timeline of infection, symptoms, and biomarkers. Shown is the chronology of viral replication in relation to clinical and biologic signs of disease, including the biomarkers used in diagnostic assays to detect chikungunya virus infection. Adapted from Weaver and Lecuit (2015) and from Suhrbier et al. (2012). Copyright Massachusetts Medical Society 2015. Reproduced with permission.

clinical symptoms than lower viral load (Dutta et al., 2014). However, other studies have reported that the viral load of symptomatic individuals does not differ with clinical presentation or co-morbidity, although it tends to be higher than in viremic asymptomatic individuals (Appassakij et al., 2013; Thiberville et al., 2013a). Several studies have also established that viral load is higher in newborns and the elderly (Laurent et al., 2007; Staikowsky et al., 2009; Thiberville et al., 2013a).

Concomitant with viremia, the most common biological abnormality is leukopenia, and in particular lymphopenia (Staikowsky et al., 2009), which is more pronounced in patients with higher viremia (Borgherini et al., 2007). Other immunological markers associated with severe disease include notably high levels of type-I IFNs, IL-1β, IL-6, MCP-1 and TNFα (Kelvin et al., 2011; Ng et al., 2009; Venugopalan et al., 2014; Wauquier et al., 2011). Debilitating polyarthralgia is reported in the large majority of symptomatic patients, although children tend to display milder arthralgia (Jaffar-Bandjee et al., 2010). Joint pain is typically polyarticular, bilateral, symmetrical and affects mainly the extremities (ankles, wrists, phalanges) but also larger joints (shoulders, elbows and knees) (Manimunda et al., 2010; Simon et al., 2007; Sissoko et al., 2010; Thiberville et al., 2013a). Joint symptoms can fluctuate in intensity, but do not usually vary anatomical location. Swelling may also occur in the interphalangeal joints, wrists, and ankles, as well as pain along ligament insertions, notably in children. Arthralgia experienced by CHIKV-infected patients closely resembles the symptoms induced by other viruses including arthritogenic alphaviruses such as RRV and BFV (Jacups et al., 2008; Suhrbier et al., 2012). Myalgia is also frequently observed, its prevalence varying from one study to another (Mohd Zim et al., 2013; Staikowsky et al., 2009; Thiberville et al., 2013a), predominantly in the arms, thighs and calves.

During the acute stage of CHIKV infection, rash occurs in 10% to 40% of cases depending on the study (Borgherini et al., 2007; Economopoulou et al., 2009). It is characterized by transient macular or maculopapular rash that involves mainly the extremities, but rarely the face, and lasts for 2–3 days. Children show a high prevalence of dermatological manifestations including hyperpigmentation, generalized erythema, maculopapular rash and vesiculobullous lesions (Thiberville et al., 2013b; Valamparampil et al., 2009).

Rare ocular complications can occur during the acute illness, or with a delay, including uveitis, iridocyclitis, and retinitis (Lalitha et al., 2007; Mahendradas et al., 2008).

Less frequently, symptoms include lymphadenopathy, pruritus, and digestive abnormalities, which are more common after viremia has resolved (Staikowsky et al., 2009; Win et al., 2010; Wu et al., 2011).

Fever usually lasts less than a week, until viremia ends. This is the time when patients mount anti-CHIKV adaptive immunity, characterized by the appearance of anti-CHIKV antibodies (Carey et al., 1969). Joint symptoms usually resolve within 1–2 weeks, but a large proportion of patients exhibit persistent or relapsing arthralgia that lasts for months or years (see below).

It is notable that disease severity may depend on hosts and virus factors. The La Réunion isolate has been shown to replicate to higher level compared to both a West African lineage strain in rhesus macaques and an Asian lineage isolate responsible for the recent Caribbean outbreak in a mouse model (Messaoudi et al., 2013; Teo et al., 2015). This may correlate with differences in terms of acute disease severity as well as prolonged symptoms prevalence associated with Indian Ocean vs. West Africa and Asia lineages, respectively.

2.2. Severe acute chikungunya in humans

Severe CHIKV disease in otherwise healthy individuals occurs mainly in the extreme ages, in elderly patients and young children. Adults with severe disease usually display underlying condition, such as diabetes, alcoholic hepatopathy, stroke, epilepsy, hypertension, or impaired renal function, which are independent risk factors for severe disease (Economopoulou et al., 2009). Severe disease can manifest as encephalopathy and encephalitis, cardiovascular and respiratory disorders, renal failure, hepatitis and myocarditis (Borgherini et al., 2007; Das et al., 2010; Economopoulou et al., 2009; Staikowsky et al., 2009).

Although CHIKV is not considered to be neurotropic, recent evidence suggests a neurological involvement in CHIKV infection, notably in infected neonates and young children and the elderly, who appear more prone to this complication. Most of the evidence on CHIKV neurotropism stem from reports from the outbreaks due to IOL CHIKV strains, over the past decade in La Réunion Island and India. Neurological presentations may include encephalitis, encephalopathy, acute flaccid paralysis, meningoencephalitis and Guillain–Barré syndrome (Economopoulou et al., 2009; Rampal and Meena, 2007; Singh et al., 2008; Wielanek et al., 2007). The analysis of a cohort of CHIKV-infected patients with CHIKV RNA- or anti-CHIKV-IgM positive cerebrospinal fluid (CSF) shows that 42% of them fulfil the International Encephalitis Consortium criteria for encephalitis (Venkatesan et al., 2013), with an age distribution curve exhibiting a U-shaped pattern with a very clear trend towards the highest incidence at the youngest age (Gérardin

et al., under revision). In young children, including neonates, neurological complications include encephalitis, seizures and acute encephalopathy.

Mother-to-child transmission of CHIKV infection was first reported during the La Réunion outbreak, as a cause of severe neonatal disease, associated with neurological acute symptoms (Gerardin et al., 2008; Gerardin et al., 2014; Gupta et al., 2015; Ramful et al., 2007). The overall prevalence of maternal-fetal transmission is actually low (0.25% after 22 weeks) (Gerardin et al., 2008), and vertical transmission is observed exclusively in near-term deliveries in the context of intrapartum maternal viremia, with a rate that reached up to 50% during the La Reunion outbreak (Gerardin et al., 2008). Whether host and viral genetic factors have an impact on mother-to-child transmission remains to be determined. The preventive role of Caesarean section on transmission is not precisely known. During the La Reunion outbreak, severe illness was observed in 53% of infected neonates and mainly consisted of neurological signs (Gerardin et al., 2008). Importantly, CHIKV infection in the perinatal period can cause severe disease with lifelong disability, as 51% of infected children exhibit global neurodevelopmental delay (Gerardin et al., 2014).

Detailed epidemiological investigations of maternal-fetal CHIKV transmission, as well as human placental and experimental in vivo animal studies have led to the conclusion that vertical contamination most probably occurs as a consequence of passive transfer of maternal blood free CHIKV infectious particles through the placental barrier via the physiological breaches that arise at the term of pregnancy and during parturition, and which are known to lead to maternal-fetal blood exchanges (Couderc et al., 2008; Gerardin et al., 2008).

Hemorrhagic complications are exceptional, if they exist at all, and should therefore lead to the consideration of alternate diagnoses, such as a co-infection with DENV, or comorbidities such as chronic hepatopathy.

CHIKV-infected patients with severe disease often require hospitalization, in the context of their advanced age and loss of autonomy. Deaths due to the infection were documented for the first time during the 2005–2006 outbreak in La Réunion (Economopoulou et al., 2009), and the most common causes of death were heart failure, multiple organ failure, hepatitis, and encephalitis. In epidemics that have occurred since 2005, the case-fatality rates were low, approximately 1 in 1000 (Borgherini et al., 2007; Gerardin et al., 2008; Lemant et al., 2008; Tandale et al., 2009). During CHIKV epidemics, a total of 260 excess deaths were reported in La Réunion island (mortality rate attributed to chikungunya 1/1000) and a total of 2944 excess deaths occurred in India (Ahmedabad) (mortality rate attributed to chikungunya 0.8/1000) (Josseran et al., 2006; Mavalankar et al., 2007; Mavalankar et al., 2008; Pialoux et al., 2007). Most of the deaths occurred in adults with underlying conditions, as well as, rarely, in neonates.

2.3. Chronic chikungunya in humans

In contrast to the other symptoms that manifest at the acute phase, joint pain may persist, and relapse, for weeks to months and even years (Ganu and Ganu, 2011; Schilte et al., 2013; Sissoko et al., 2009). Long-lasting symptoms are typically not observed in dengue fever, although asthenia can be intense in the days to weeks following the dengue fever. Chronic joint pain following chikungunya disease was first published in 1979 (Fourie and Morrison, 1979) and has been massively reported during all recent epidemics that have occurred since 2005 in the Indian Ocean notably in the Island of La Réunion (Borgherini et al., 2008; Hoarau et al., 2010; Javelle et al., 2015; Marimoutou et al., 2015; Schilte et al., 2013; Sissoko et al., 2009; Thiberville et al., 2013a) or in metropolitan France for imported cases (Couturier et al., 2012; Larrieu et al., 2010; Simon et al., 2007), in India or Southeast Asia (Ganu and Ganu, 2011; Manimunda et al., 2010; Narsimulu and Prabhu, 2011; Win et al., 2010), in Italy (Moro et al., 2012) and more recently in the Caribbean (Foissac et al., 2015; Miner et al., 2015). Although the overall proportion of patients with chronic symptoms decreases over time after CHIKV disease onset (from 100% to 88% during the first 6 weeks, to less than 50% after 3–5 years, with variable findings depending on the studies), the time required for a complete healing of all symptoms is still uncertain, as some infected individuals remain symptomatic for years post-infection (Ganu and Ganu, 2011; Schilte et al., 2013; Sissoko et al., 2009).

Chikungunya chronic disease is characterized by persistent or relapsing arthralgia usually located at the same joint sites that were affected during the acute phase, and may mimic rheumatoid arthritis (chronic inflammatory, and rarely erosive and even deforming polyarthritis) (Ganu and Ganu, 2011; Javelle et al., 2015; Schilte et al., 2013).

In a recent 6-year retrospective study on a cohort of patients in La Réunion, two main categories of post-chikungunya persisting rheumatic and musculoskeletal disorders were distinguished (Javelle et al., 2015): patients without previously defined arthritis who correspond to 27% of patients, and who present with current musculoskeletal disorders (loco-regional or diffuse), and patients with non-crystalline polyarthritis who represent 70% of patients fulfilling the diagnostic criteria of rheumatoid arthritis, spondyloarthritis or undifferentiated polyarthritis. The latter were refered to as patients with chronic inflammatory rheumatism (CIR). Among them, a minority (16%) had pre-existing CIR that immediately exacerbated after CHIKV infection, while all the other developed CIR following CHIKV infection. As CIR may progress in a potentially destructive disease, early disease management by a specialized medical team is important. Notably, early treatment with anti-inflammatory and immunosuppressive drugs such as methotrexate might prevent joint damage, although their safety and efficacy remain to be validated in this context (Javelle et al., 2015).

It is noteworthy that joints that are already damaged by underlying disorders, such as osteoarthritis seem to constitute preferential sites for long-term pain (Sissoko et al., 2009). Of note, the likelihood of developing persistent arthralgia is highly dependent on age (Javelle et al., 2015; Schilte et al., 2013).

This chronic disease has been reported to be associated with detectable IL-17 and elevated serum levels of IL-6 and granulocyte macrophage colony-stimulating factor in patients at 2–3 months after illness onset (Chow et al., 2011). However, in a study conducted in patients with chronic symptoms up to 36 months after the acute phase, no systemic biomarkers associated with chronic arthralgia nor biological markers typically found in autoimmune or rheumatoid diseases were reported (Schilte et al., 2013).

Although the chronic disease generally causes less debilitating pains than the acute disease, many patients still have a pronounced reduction in movement and quality of life and require long-term treatment (Couturier et al., 2012; Moro et al., 2012; Rosario et al., 2015; Schilte et al., 2013).

3. Experimental CHIKV infection in animal models mimicking some features of human chikungunya

Animal models for CHIKV infection, including mice and non-human primates have been used to study CHIKV-associated pathologies (Gasque et al., 2015). A zebrafish animal model has also been developed and used to visualize CHIKV infection and innate host responses to infection (Palha et al., 2013). However,

most studies have been conducted in mice, including investigation on CHIKV tissue and cell tropisms, as well as host responses to infection.

Adult immunocompetent mice (wt mice) do not develop clinical signs following intraperitoneal, intradermal or intravenous virus inoculations (Teo et al., 2012). However, adult or 14-day-old C57BL/6 mice develop viremia and pathological changes after CHIKV inoculation via the subcutaneous route in the footpad, which are restricted to lesions in muscles of the infected foot, leg swelling, edema with evidence of arthritis and tenosynovitis during the acute phase (Gardner et al., 2010; Morrison et al., 2011). Moreover, newborn and 14-day-old outbred mice, and 8–9 day-old C57BL/6 are susceptible to CHIKV infection, either through the subcutaneous route in the loose skin of the back, or in the thorax via the intradermal route, respectively (Couderc et al., 2008; Ziegler et al., 2008). They develop viremia and skeletal muscle weakness that can be fatal in the case of younger mice and histopathological analysis of the affected limb reveals myositis with necrosis.

Adult mice with a complete deficiency in the type-I interferon receptor ($IFNAR^{-/-}$) develop a severe infection after intra-dermal CHIKV inoculation and to a lesser extent after ocular inoculation (Couderc et al., 2008; Couderc et al., 2012). The disease is characterized by muscle weakness of the limbs and lethargy, and is often fatal. Interestingly, adult mice with a partially abrogated type-I interferon receptor deficiency ($IFNAR^{+/-}$) develop a mild disease without viremia but with a low level of replication in muscles and joints, indicating that the gene copy number of the type-I IFN receptor influences viral load and tissue distribution, as well as the severity of the disease (Couderc et al., 2008). Therefore, young age and inefficient type-I IFN signaling are major factors of susceptibility to CHIKV severe disease in mice.

In contrast to adult wt mice, non-human primates are susceptible to CHIKV infection and this susceptibility has been reported since 1967, when rhesus monkey were reported to be susceptible to experimental infection with CHIKV as evidenced by a febrile reaction and high levels of circulating virus (Binn et al., 1967). More recently, studies performed in cynomolgus a (*Macaca fascicularis*) and rhesus (*Macaca mulatta*) macaques experimentally infected with CHIKV have shown that inoculation via the intravenous route leads to systemic infection, with viremia levels of up to 10^8 PFU/mL, even if the infectious dose inoculated is low (10 PFU) (Labadie et al., 2010; Messaoudi et al., 2013). With the highest infectious dose (10^8 PFU), monkeys developed clinical neurological disease characterized by meningo-encephalitis (Labadie et al., 2010). At the peak of viremia, leukopenia including lymphopenia is observed, similarly to lymphopenia observed in humans at the acute phase of the disease, as well as markers of type-I IFN response, inflammation, and cell immune activation (Labadie et al., 2010). In macaques, CHIKV targets joints, secondary lymphoid organs, liver, and, to a lesser extent, muscle and skin. Interestingly, long-term CHIKV infection can be observed, mainly in secondary lymphoid organs in cynomolgus macaques (Labadie et al., 2010) and in aged rhesus macaques (Messaoudi et al., 2013). Thus, the CHIKV-infected monkey provides an animal model to study these features of acute but also long-term evolution of chikungunya.

The lower susceptibility of mice as compared to humans or monkeys may involve species-specific factors. Actually, it has been shown that the human autophagy receptor NDP52 interacts with the CHIKV nonstructural protein nsP2, thereby promoting viral replication in human cell cultures, whereas the NDP52 mouse ortholog is unable to bind to nsP2 and to promote CHIKV infection in mouse cell cultures (Judith et al., 2013). Thus, the absence of the proviral effect of NDP52 in the mouse may contribute to its lower permissiveness to CHIKV relative to humans. Whereas it is clear that an increased neonatal susceptibility is also observed in humans, the relevance of a type-I IFN defect and autophagy receptor NDP52 as a basis for severe infection in humans remains to be demonstrated.

Studies performed in animal models experimentally infected with CHIKV have also contributed to decipher the pathophysiology of the disease (see below).

4. Resolved and pending questions regarding the pathophysiology of CHIKV infection

Whereas the pathophysiology of infection with other alphaviruses, such as SFV and SINV has been studied for several decades, the pathophysiology of CHIKV infection has been investigated only recently. The studies performed in naturally infected human and experimentally infected animal models have provided clues to the cell and tissue tropisms of CHIKV, as well as host factors that control infection during the acute phase of infection.

4.1. Cell and tissues tropisms of CHIKV

4.1.1. Acute phase of chikungunya disease

The cell and tissue tropisms of CHIKV at the acute phase of the disease have been investigated in humans and in animal models. Moreover, studies on CHIKV infection of cultures of primary cells and cell lines have also contributed to the deciphering of the cell biology of CHIKV infection.

In $IFNAR^{-/-}$ mice, CHIKV initially targets the liver, causes high viremia and replicates in connective tissues, particularly in the epimysium (also called muscle fascia) of skeletal muscle, in myo-tendinous insertions of muscle and in joint capsules, and to a lesser extent in the perimysium and endomysium of skeletal muscles (Couderc et al., 2008). Data in humans and infected muscle and joint tissues of mice showed that fibroblasts constitute major target cells of CHIKV at the acute phase of the infection (Fig. 1). In mouse skeletal muscle, CHIKV can also be detected, albeit rarely, in satellite cells, consistent with a study performed on human material (Ozden et al., 2007). Thus, CHIKV infection pathophysiology largely resembles to that of other arthritogenic alphaviruses, as the connective tissues of joints and skeletal muscles, and tendons are also the sites of replication of RRV and SINV (Heise et al., 2000; Morrison et al., 2006). Of note, both joint and muscle connective tissues contain a high amount of nociceptive nerve-endings, and their stimulation upon infection may account for the muscle and joint pain characterizing disease caused by alphaviruses associated with muscle and joint pathology (Couderc et al., 2009). The pain triggered by joint mobilization may also result from the infection of musculo-tendinous insertions surrounding them.

Viral cell tropism in infected peripheral tissues of C57BL/6 mouse neonates, including muscle and joints, is similar to that of adult $IFNAR^{-/-}$ mice, with a pronounced tropism for fibroblasts (Couderc et al., 2008). A notable difference is the presence of severe necrotic myositis consistent with myofiber necrosis and inflammation manifested by the presence of infiltrates of lymphocytes and monocytes/macrophages. Similar data are found in young outbread mice (Ziegler et al., 2008). Interestingly, in human adult muscle biopsies, myositis together with inflammatory infiltrates mainly consisting of monocytes/macrophages and T cells have been reported (Ozden et al., 2007). Similarly to CHIKV in mouse neonates, RRV has been shown to induce myositis in mice (Heise et al., 2000; Morrison et al., 2006; Ryman et al., 2000).

In the case of severe disease in mice, viremia is high and CHIKV also disseminates to other tissues, including skin, eye and the central nervous system (CNS) (Fig. 1). CHIKV targets fibroblasts of

deep dermis in the skin, as well as fibroblasts in the eye, including those of corneal and scleral stroma, corneal endothelium, ciliary body smooth muscle stroma, iris and those between ocular muscle fibers (Couderc et al., 2008; Couderc et al., 2012). In humans, CHIKV antigens have been found in fibroblasts of the same sites (Couderc et al., 2008; Couderc et al., 2012). Uveitis is due to inflammation of the uvea (iris, ciliary body, and choroid), and histological data in CHIKV-infected ocular tissues may therefore provide a virological basis for uveitis, the main ocular manifestation associated with CHIKV infection (Mahendradas et al., 2008; Rajapakse et al., 2010).

Together, these data demonstrate that infection of peripheral tissues responsible for symptoms in human in the context of acute CHIKV infection, i.e. joints, muscle, skin and eye, is restricted mainly to conjunctive tissues and the fibroblast as predominant target cell of CHIKV during acute infection. They also reveal that CHIKV and other arthritogenic alphaviruses share some tissue tropism similarities.

In vivo findings are consistent with the in vitro observation that human and mouse primary muscle fibroblasts, as well as primary human skin fibroblasts are susceptible to CHIKV infection (Couderc et al., 2008; Ekchariyawat et al., 2015). Fibroblasts derived from other tissues have also been shown to be permissive to CHIKV, including human lung fibroblasts, primary human foreskin fibroblasts, and mouse embryonic fibroblasts (Schilte et al., 2010; Sourisseau et al., 2007). These cells, as well as primary human skin fibroblasts, produce high level of IFNβ triggered by MAVS activation upon CHIKV infection (Ekchariyawat et al., 2015; Schilte et al., 2010). It has been shown that infection of primary human skin fibroblasts triggers, in addition to IFNβ, enhancement of IL-1β expression, maturation of caspase-1 and expression of the inflammasome sensor AIM2 (Ekchariyawat et al., 2015). Moreover silencing of caspase-1 enhances viral replication. This suggests that CHIKV-infected skin fibroblasts may contribute to a pro-inflammatory and antiviral microenvironment (Ekchariyawat et al., 2015).

The molecular basis for the prominent in vivo tropism for fibroblasts is unknown and may indicate that fibroblasts could be, relative to other cell types, either (i) in a hyper-permissive status regarding CHIKV entry/replication, and/or (ii) in a hypo-sensitive status to type-I IFN-mediated viral interference, making them a target of choice for CHIKV (Couderc et al., 2008). However, a study has shown that two human fibroblast cell lines with different susceptibility to CHIKV infection failed to show that differences in the primary type-I IFN and IFN-stimulated genes (ISG) responses to CHIKV were responsible (Thon-Hon et al., 2012). Alternatively, CHIKV cell-to-cell dissemination in fibroblasts may be dependent on the particular structure of connective tissues of dermis, joint capsules and muscles, that have in common the property to form a reticular network of cells interconnected by gap junctions (Langevin et al., 2004).

Beside fibroblasts, monocytes/macrophages are another cell type possibly targeted by CHIKV. Actually, CHIKV antigens were detected in blood monocytes of acutely infected patients and monkeys early after infection (Her et al., 2010; Roques and Gras, 2011). Primary human monocytes and macrophages could also be infected with CHIKV, although with low efficiency (Sourisseau et al., 2007; Teng et al., 2012) and in vivo, viral antigens were detected in monkey macrophages during the viremic phase of infection but also at later time points (6 weeks pi) (Labadie et al., 2010), suggesting that they may either be a site of CHIKV replication, or of antigen clearance. These findings suggest that monocytes/macrophages might contribute to CHIKV physiopathology, although their contribution to viral load remains to be determined.

The susceptibility of monocytes/macrophages to CHIKV infection has been further investigated using in vitro models. A study revealed that CHIKV infection of human macrophage cell lines could be significantly increased by the presence of CHIKV-apoptotic blebs in the culture medium (Krejbich-Trotot et al., 2011). Another study showed that the murine macrophage cell line Raw264.7 can be infected by CHIKV but that only a subset of cells is susceptible to infection and produce infectious particles, whereas the others appears to be refractory to infection (Kumar et al., 2012).

Altogether these studies on monocytes/macrophages highlight the lower susceptibility of these cells to CHIKV, as compared to fibroblasts, which may involve cell specific host cell factors determining the susceptibility and/or resistance to infection. Compared to monocytes, primary cultures of B and T cells were found not to be susceptible to CHIKV infection in vitro (Her et al., 2010; Sourisseau et al., 2007; Teng et al., 2012).

Other in vitro studies have highlighted the specific susceptibility or resistance of cells to CHIKV infection. CHIKV replicates in human muscle satellite cells but fails to infect differentiated myotubes (Ozden et al., 2007), according to in vivo findings. Interestingly, human primary keratinocytes can be infected by CHIKV but viral RNA synthesis is impaired such as de novo viral particle are not produced, suggesting an intracellular block of CHIKV replication in human keratinocytes (Bernard et al., 2015). According to this finding, the replication of CHIKV in keratinocytes in vivo has thus far never been reported. In vitro, mouse embryonic stem cells are susceptible to CHIKV infection and sense type I IFNs, but to a lower extent than CHIKV-infected fibroblasts. Moreover, they are deficient in type I-IFN expression, as compared to CHIKV-infected fibroblasts (Wang et al., 2014). Given the relative deficiency of stem cells to produce and respond to type-I IFN, they may constitute an important target for CHIKV in vivo, and may have a relevance for the long term consequences of infection.

4.1.2. Severe acute chikungunya disease

In the CNS of experimentally infected highly susceptible mice, CHIKV targets the choroid plexuses, meningeal and ependymal envelopes, but brain microvessels and parenchyma are spared in neonates, adult IFNAR$^{-/-}$ mice, as well as in adult infected monkeys (Couderc et al., 2008; Ziegler et al., 2008). Primary mouse choroid plexus epithelial cells were highly susceptible to infection, while primary mouse brain microvessel endothelial cells were fully resistant to CHIKV infection (Couderc et al., 2008). In humans, CHIKV and anti-CHIKV IgMs have been detected in the CSF of neonates and adult patients with CNS symptoms (Grivard et al., 2007). Altogether, these data suggest that CHIKV may infect and cross the blood–brain barrier at the choroid plexus and leptomeningeal levels and then infects CNS envelopes. In monkeys, high levels of cytokines are found to be associated with encephalopathy (Labadie et al., 2010). Thus, the cytopathic effects induced in infected cells of brain envelopes and the host responses triggered, may affect underlying neuronal cells, leading to the CNS signs and symptoms associated with neurologic symptoms. In contrast to adult animals, a recent study has shown that CHIKV infects parenchymal cells in neonatal/suckling mice but the nature of these cells is unknown (Dhanwani et al., 2011). A defective host response may contribute to CHIKV neurotropism in neonates, as well as to the generally higher susceptibility of neonates to severe CHIKV infection, in agreement with the fact that the neonatal immune response is quantitatively and qualitatively distinct from that of adults (Adkins et al., 2004). In vitro studies show that CHIKV replicates in mouse astrocytes and oligodendrocytes, and with lower efficacy in neuronal cells but fails to infect microglia (Das et al., 2015). Further studies will be needed to fully decipher CHIKV tissue and cell neurotropism. In contrast, it is well established that New World alphaviruses cause encephalitis in humans and in animal models as a consequence of viral invasion of the brain microvessels and parenchyma (Ferguson et al., 2015; Zacks and Paessler, 2010).

Mother-to-child CHIKV transmission has been studied in experimental infection of pregnant animals. In pregnant viremic macaques and mice, transplacental transmission is not observed (Couderc et al., 2008; Chen et al., 2010). Investigation of mouse and human placentas from viremic mothers have shown that, in contrast to SFV and RRV, CHIKV does not directly infect trophoblastic cells and is therefore probably transmitted to neonates through maternal–fetal blood exchange during delivery, accounting for the low frequency of mother-to-child transmission before near term delivery (Aaskov et al., 1981a,b; Couderc et al., 2008; Milner and Marshall, 1984).

4.1.3. Chronic phase of chikungunya disease

The pathophysiology of CHIKV chronic disease remains poorly understood and, to date, no animal model fully reproduces the chronic joint syndrome associated with many cases. In humans, patients with chronic CHIKV-induced arthralgia often have persistent virus-specific IgM (Borgherini et al., 2008; Malvy et al., 2009), that could result from continued exposure to CHIKV antigen. One study has provided evidence for persistence of viral antigen and RNA in synovial tissue from a patient with chronic arthralgia for 18 months after CHIKV infection (Hoarau et al., 2010). In animal models, persistence of viral RNA has been shown both in mice and in monkeys. In mice, viral RNA can be detected in joint-associated tissues for at least 16 weeks following footpad injection and is associated with histopathological evidence of joint inflammation (Hawman et al., 2013; Poo et al., 2014b). Interestingly, studies in CHIKV-infected macaque have demonstrated that viral RNA persists in lymphoid organs and liver and, to a lesser extent, in muscle and joints of macaques, and identified macrophages as the main cellular reservoirs during the late stages of CHIKV infection (Chen et al., 2010; Labadie et al., 2010), suggesting that macrophages play also a role in chronic disease. Viral RNA persistence in both one human case and CHIKV animal models raises the question of the role of CHIKV RNA in joint inflammation and injury, as it has been shown that double stranded RNA, a product of viral genome replication, is arthrithogenic (Zare et al., 2004).

In addition to IgM, patients with chronic joint pains can display elevated IL-6 levels (Chaaitanya et al., 2011; Chow et al., 2011; Reddy et al., 2014). For RRV, it has been shown that infection of osteoblasts results in increased of IL-6 together with a change of the ratio of Receptor Activator of Nuclear Factor-KappaB Ligand (RANKL) to osteoprotegerin (OPG), which have been implicated in bone disease including arthritis and osteoporose (Chen et al., 2014). In mouse models, infectious RRV is detected in bones and a reduction of bone volumes is observed in association with a disruption of the ratio of RANKL/OPG. Importantly, the bone loss caused by RRV infection in mice, as well as the changes in the ratio RANKL/OPG are prevented by IL-6 inhibition (Chen et al., 2014). These findings suggest a mechanism involving direct infection of osteoblasts to explain joint pathologies during infection with RRV and possibly CHIKV (Burt et al., 2014). As for RRV, pre-existing arthritis is exacerbated by CHIKV infection (Javelle et al., 2015) and the inflammatory response during alphavirus infection in the joint is similar to that in rheumatoid arthritis, with a similar pattern of leukocytes infiltration, cytokine production and complement activation (Burt et al., 2012; Nakaya et al., 2012; Scott et al., 2010). Deciphering the viral and immune mechanisms leading to joint pathologies is a health concern, notably in the case of pre-existing arthritis given the relatively high incidence of bone disease in the general population.

4.2. Host response to CHIKV infection

Studies in humans and animal models have shown that innate immunity, and particularly the type-I IFN response, is crucial for restricting virus replication during the acute phase of infection. Moreover, as mentioned above, antibodies against CHIKV are present in the serum of infected humans at the end of viremia.

4.2.1. Innate response to CHIKV infection

Alphaviruses, including CHIKV, are long known to be strong inducers of type-I IFN and sensitive to type-I IFN responses (Farber and Glasgow, 1972; Glasgow et al., 1971; Grieder and Vogel, 1999). More recent studies in patients in La Réunion also reported that CHIKV infection elicited high levels of IFN-α in the serum and its concentration correlated with viral load (Schilte et al., 2010; Wauquier et al., 2011). Similar to human patients, the acute disease in monkeys is associated with substantially increased type-I IFN concentration in the plasma (Labadie et al., 2010). As in mammals, CHIKV infection triggers a strong type-I IFN response, critical for survival in CHIKV-infected zebrafish (Palha et al., 2013).

Recently, the roles of type-I IFN in CHIKV infection and its related antiviral pathways have been deciphered. As mentioned above, adult IFNAR$^{-/-}$ mice are fully susceptible to sever CHIKV infection, in contrast to adult wt mice. Interestingly, it has been shown that this absence of susceptibility upon intradermal infection of wt mice is due to the very early control of CHIKV infection by type-I IFN at the site of injection in the skin. Actually, infection is rapidly controlled by IFN-β secreted locally by infected dermal fibroblasts, which leads to antiviral response at the site of injection (Schilte et al., 2010). It is notable that no other cell type, including hematopoietic cells, is infected at the injection site in wt adult mice.

The role of type-I IFN in CHIKV pathogenesis has been investigated further in humans and in mouse models, as well as in human cells. In mice, it has been demonstrated that CHIKV is controlled by the direct action of type-I IFN on nonhematopoietic cells (Schilte et al., 2010), and nonhematopoietic cell-derived type I-IFN is sufficient to control CHIKV infection (Schilte et al., 2012). Moreover, the production of type I-IFN by nonhematopoietic cells acts via an MAVS-dependent signaling pathway likely triggered by activation of host sensors (RIG-I and/or MDA5) for CHIKV RNA in infected fibroblasts (Rudd et al., 2012; Schilte et al., 2010; Schilte et al., 2012; White et al., 2011). It has also been shown that IRF-3 or IRF-7 expression in either hematopoietic or nonhemotopoietic cell compartments is capable of inducing an antiviral response. Moreover, IRF-3 or IRF-7 signaling is sufficient to control CHIKV in adult mice, whereas both transcription factors are required in mouse neonates (Schilte et al., 2012). These findings indicate that IRF-3 and IRF-7 play an essential role in the control of neonatal CHIKV infection in mice and highlight an age-dependent redundancy for IRF-3 or IRF-7 in adult but not newborn animals.

Type-I IFN is able to trigger the activation of a specific signal transduction pathway leading to induction of ISGs that are responsible for the establishment of an antiviral state. ISGs that have been found to exert an antiviral role against CHIKV in vitro and/or in vivo include ISG15, ISG20, P56, ZAP, OAS3 and Viperin (Brehin et al., 2009; Lenschow et al., 2007; MacDonald et al., 2007; Werneke et al., 2011; Zhang et al., 2007).

The role of monocytes/macrophages in CHIKV disease has also been investigated in the acute phase of the disease. Studies with non-human primate and mouse models also suggest that viral replication in joint tissues leads to the recruitment of inflammatory cells, with monocytes, macrophages, and natural killer cells being the major inflammatory cell types (Gardner et al., 2010; Labadie et al., 2010). Consistent with the role of monocytes/-macrophages in inflammation during the acute disease are studies showing that macrophage depletion in mice reduces foot swelling induced by injection of CHIKV in the footpad. Consistent with this observation, treatment with Bindarit, an inhibitor of monocyte

chemoattractant protein-1 (MCP-1) chemokine (CCL2) production, blocks monocytes recruitment and reduces joint inflammation in mice (Chen et al., 2015; Gardner et al., 2010; Rulli et al., 2011). However, the recruitment of monocytes/macrophages also appears to be critical for preventing excessive pathology and resolving inflammation, as CHIKV-infection in CCR2$^{-/-}$-deficient mice with a defective CCL2 receptor results in more severe disease due to an excessive recruitment of neutrophils rather than monocytes/-macrophages to the inflamed joint (Poo et al., 2014a).

These data provide evidence that monocytes/macrophages are involved in the pathophysiology of CHIKV acute disease. As mentioned above, some of them seem to be targeted by CHIKV. Further studies will be required to clarify the relationship between their role as target cells and as innate immune cells together with their role in inflammation during the acute phase of the disease.

Autophagy has been shown to be an innate mechanism involved in host response to CHIKV infection. Actually, Atg16L(HM) mice, which display reduced levels of autophagy, showed a higher sensitivity to CHIKV-induced apoptosis and exhibited increased lethality, suggesting that inducers of autophagy inhibits apoptosis and may limit the pathogenesis of acute chikungunya (Joubert et al., 2012).

Besides innate response, CHIKV infection also leads to a protective adaptive immunity.

4.2.2. Adaptive immunity to CHIKV infection

Anti-CHIKV immunoglobulin M (IgM) and immunoglobulin G (IgG) are detected in the sera of infected patients during the acute phase of disease (Kam et al., 2012; Mohd Zim et al., 2013; Nitatpattana et al., 2014; Panning et al., 2008). These antibodies exhibit a high in vitro neutralizing activity and in vivo studies have demonstrated the importance of anti-CHIKV neutralizing antibodies in the protection against CHIKV infection (see below, *Immunotherapy*). The protective role of antibody in CHIKV infection is also illustrated by the report that infected mice deficient for TLR3 signaling develop a more severe disease than wt mice and synthesize anti-CHIKV antibodies that exhibit a lower in vitro neutralization potency than those generated in wt mice (Her et al., 2015).

The role of B and T cells has been investigated in Rag1$^{-/-}$ mice, which lack T and B cells. After infection in the footpad, Rag1$^{-/-}$ mice display higher viral levels in a variety of tissues than wt mice, suggesting that adaptive immunity controls the tissue specificity and helps clear CHIKV infection (Hawman et al., 2013). The role of B cells was also explored in B cell deficient (μMT) knockout mice infected with CHIKV in the footpad. In these animals, viremia persisted for over a year, indicating a direct role for B cells and antibody in mediating CHIKV clearance (Lum et al., 2013; Poo et al., 2014b). Moreover, these infected mice exhibited a more severe disease than wt mice during the acute phase. The roles of T cells were explored in adult mice deficient for T cells and infected in the footpad. Interestingly, it was found that CHIKV-specific CD4^{+} but not CD8^{+} T cells are essential for the development of joint swelling without any effect on virus replication and dissemination, suggesting T cells are involved in inflammation (Hawman et al., 2013; Teo et al., 2013). This corroborates observations made from human muscle biopsies where T cells, but not B cells, were detected (Ozden et al., 2007).

The importance of B and T cells in protection against CHIKV infections has also been demonstrated by vaccine studies (Mallilankaraman et al., 2011). Actually, vaccination against CHIKV is able to induce a strong CD8+ T cell-mediated cellular response as well as a humoral response that protects mouse and nonhuman primate models against a lethal challenge (Mallilankaraman et al., 2011; Partidos et al., 2011).

Finally, the role of host age on the T and B cell responses has been investigated in rhesus macaques. Interestingly, aged animals have delayed and/or reduced T and B cell immunity compared to adult animals (Messaoudi et al., 2013). Moreover, while adult animals are able to control viral infection, aged animals show persistent virus in the spleen. These data support clinical findings of CHIKV susceptibility in elderly humans and provide evidence that an effective T and B cell responses against the virus are required for preventing persistent CHIKV infection.

4.2.3. Immunotherapy

The protective effect of passive immunization against alphaviruses, including Venezuelan Equine Encephalitis virus, SINV and SFV, was long ago demonstrated in mouse models a long time ago (Boere et al., 1983; Mathews et al., 1985; Schmaljohn et al., 1983). In recent years, passive immunization has been investigated in mouse models susceptible to CHIKV infection. The first study reporting the use of passive immunization against CHIKV was performed with human polyvalent antibodies purified from human plasma donors in the convalescent phase of CHIKV infection (Couderc et al., 2009). These antibodies exhibited strong neutralizing activity in vitro and had a full protective efficacy in highly susceptible mouse models: adult IFNAR$^{-/-}$ mice and neonatal C57BL/6 mice. Moreover, they displayed a therapeutic efficacy in these animal models when administrated after infection. Similarly, purified polyclonal antibodies from monkeys immunized with a CHIKV virus-like particle vaccine protected IFNAR$^{-/-}$ mice from CHIKV-induced disease and death (Akahata et al., 2010).

As for neutralizing polyclonal antibodies, human and mouse neutralizing monoclonal antibodies have been shown to protect mice against CHIKV. Human neutralizing monoclonal antibodies directed against E2 or E1 significantly delay lethality of CHIKV-infected mice, both in prophylactic or therapeutic settings (Fong et al., 2014; Fric et al., 2013). Similarly, a human neutralizing monoclonal antibody directed against E2 protein is able to prophylactically protect adult C57BL/6 mice from viremia and foot swelling, and to therapeutically protect neonatal C57BL/6 mice from death (Selvarajah et al., 2013). In other studies, mouse neutralizing antibodies directed against the E1 or E2 protein have been shown to provide both prophylactic protection from viremia and foot swelling of adult C57BL/6 mice and from CHIKV-induced lethality in adult IFNAR$^{-/-}$ mice (Goh et al., 2013; Pal et al., 2013). Interestingly, combinations of two neutralizing monoclonal antibodies administrated after CHIKV infection completely prevent mortality of IFNAR$^{-/-}$ mice.

Altogether, these studies suggest that passive immunization may constitute an effective medical intervention for humans with a known exposure to CHIKV who are at risk for severe disease. This prophylaxis approach could thus be recommended especially during birth for neonates born to viremic mothers, who are at high risk of developing severe infection (Gerardin et al., 2008). The protective role of anti-CHIKV hyperimmune human intravenous immunoglobulins in neonates exposed to a high risk of severe form of CHIKV infection is currently under clinical investigation (see ClinicalTrials.gov number NCT02230163).

These data on the protective role of neutralizing antibodies against CHIKV disease should be taken into account in the design of an efficient vaccine against CHIKV.

5. Conclusions

Repeated, massive CHIKV outbreaks of the last decade have elucidated many new facets of CHIKV infection, including detailed clinical analysis of chronic debilitating arthragia and severe disease. Chikungunya can no longer be considered as a purely benign, self-limited disease. Studies performed in patients and animal models have provided the first data on the pathophysiology of

infection and have shown the similarities and differences with other alphavirus, as well as with dengue virus infection. However, many questions remain to be resolved, particularly the susceptibility and the role of immune cells in the acute and chronic disease and the neurotropism of CHIKV infection. Increased basic and translational research, with access to tissues and cells from infected patients, will be key to answer these questions and identify all the host and viral factors involved in the susceptibility of the host, tissue and cell to CHIKV. This research will allow us to better prevent and treat the chronic and/or severe diseases caused by this emerging alphavirus.

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