Chikungunya virus pathogenesis: From bedside to bench.

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Highlights

- Human chikungunya typically manifests as acute fever and joint pain

- Chikungunya can besevere, and associated with encephalitis, notably in neonates

- Chikungunya virus can cause chronic joint pain, and may induce arthritis

- Chikungunya virus infects fibroblasts in joints and muscles

- Type-I interferon sensing by non-myeloid cells is key to the control of chikungunya virus infection

Summary

Chikungunya virus (CHIKV) is an arbovirus transmitted to humans by mosquito bite. CHIKV has caused a decade ago a major outbreak in the Island of the Indian Ocean, and then reached India and South East Asia. More recently, CHIKV has emerged in the Americas, first reaching the Caribbean and now extending to Central, South and North America. CHIKV is therefore considered as a major public health and economic threat. CHIKV causes febrile illness typically associated with debilitating joint pains. In rare cases, CHIKVmay also causes 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 symptomsassociated with CHIKV in human. It also illustrates how the analysis of human cohorts clinical and biological data and the development of animal and cellular models of infectionas helped identify the tissue and cell tropisms of the virus and decipher host responsesin benign, severe or persistent CHIKV-associated disease.Please add the statement indicating that this is part of the symposium

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, mostlyfound in the New World and that includes western equine encephalitis and Venezuelan equine encephalitis viruses(for review see[44]). Sindbis virus (SINV), which is geographically restricted to the Old World, is howeverphylogenetically closer to the New World subgroup[44].Upon mosquito bite, CHIKV inducesan acute febrile illness typically accompanied by severe arthralgia, which can last and relapse for weeks to months. CHIKV has been the causeofseveral 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 fromAfrica, 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 CHIKVoriginating from Asia has emergedthe Caribbean and spread to South, Central and North America[140, 141].

Here we reviewthe current knowledge on CHIKV infectionmainly obtained from the analysis ofcohorts of human patients and experimental animal models.

1. Clinical presentation of chikungunya in human: 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[13, 87, 125, 126, 133, 141]). Its symptomsare similarto 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. 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 usuallyself-limited, non-fatal, with fever resolving within a fewdays. However, since the Indian Ocean outbreak in 2005–2006[115], the information available about the clinical characteristics of the human disease has significantly increasedwith the detailed clinicalstudy of cohorts of CHIKV-infected patients, notably in the Island of La Réunion, a French overseas department[124]. Previously unreported severe forms of CHIKV infection were observed, as well as maternal-fetal transmission[28, 39, 102]. 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 with possibilities of relapses,leading to arthritis and some cases destructive rheumatism, with pathogenesis has yet to be fully understood[101].

Chikungunya fever in humans

The incubation period endswitha sudden onset of high fever (>39°C), back pain, myalgia, associated tosevere and incapacitating arthralgia, together with headaches, photophobia, and rash (for a review see above).The onset of fever coincides with viremia, and bloodviral load can rapidly reach up to 10^9 viral genome copies per milliliter[96, 124]. Viral replication triggers the activation of innate immune responses, the hallmark of which is the production of type I interferons (IFNs)[116].

Apositive correlation between the intensity of viremia and acute illnesshas been observed. Actually higher viral loads have been found in hospitalized patients with comorbidity than those without[124] and is most often associated with clinical symptoms than lower viral load[27]. However, other studies have reported that the viral load of symptomatic individuals doesnot differ with clinical presentation or co-morbidity, although ittends to be

higher than in viremic asymptomatic individuals[5, 132].Several studies have alsoestablished that viral load is higher in newborns and the elderly[67, 124, 132].

Concomitant with viremia, the most common biological abnormality is leukopenia, and in particular lymphopenia[124], whichis more pronounced in patients with higher viremia[9].Other immunological markers associated with severe disease includenotably high levels of type-I IFNs, IL-1β, IL-6, MCP-1 and TNFα[60, 90, 137, 139].Debilitating polyarthralgia isreported in the large majority of symptomatic patients, although children tend to displaymilder arthralgia[54]. Joint pain is typicallypolyarticular, bilateral, symmetrical and affects mainly the extremities (ankles, wrists, phalanges) but also larger joints (shoulders, elbows and knees)[75, 119, 122, 132].Joint symptoms can fluctuate in intensity, but do not usually varyanatomical 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 includingarthritogenic alphaviruses such as RRV and BFV [53, 126].Myalgia is alsofrequentlyobserved, its prevalence varying from one study to another[83, 124, 132], predominantly in the arms, thighs and calves.

During the acute stage of CHIKV infection, rash occurs in 10% to40% of cases depending on the study[9, 28]. Itis characterized by transient macular or maculopapular rash that involves mainlythe 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[133, 135].

Rare ocular complications can occur during the acute illness, or with a delay,including uveitis, iridocyclitis, and retinitis[64, 72].

Less frequently, symptoms include lymphadenopathy, pruritus, and digestive abnormalities, which are more common after viremia has resolved[124, 145, 146].

Fever usually lasts less than a week, until viremia ends.This is the time whenpatients mountanti-CHIKV adaptive immunity,characterized by the appearance of anti-CHIKV antibodies[14].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 botha West African lineage strain in rhesus macaquesand an Asian lineageisolate responsible for the recent Caribbeanoutbreak in a mouse model [80, 131]. This may correlate with differences in terms of acute disease severity as well as prolongedsymptoms prevalence associated withIndian Ocean*vs*. West Africa and Asia lineages, respectively.

Severe acute chikungunya in humans

Severe CHIKVdisease in otherwise healthy individuals occurs mainly in the extreme ages, in elderly patients and young children.Adults with severe diseaseusually display underlying condition, such as diabetes, alcoholic hepatopathy, stroke, epilepsy, hypertension, or impaired renal function, which are independent risk factors for severe disease[28]. Severe disease can manifest as encephalopathy and encephalitis, cardiovascular and respiratory disorders, renal failure,hepatitis and myocarditis[9, 24, 28, 124].

Although CHIKV is not considered to beneurotropic, recent evidence suggests a neurological involvement in CHIKV infection,notably ininfected neonates and young children and the elderly, who appearmore prone to this complication. Most of the evidenceon CHIKV neurotropism stemfrom reports fromthe outbreaks due to IOL CHIKV strains, overthe past decade in La Réunion Island and India. Neurological presentationsmay include encephalitis, encephalopathy, acute flaccid paralysis, meningoencephalitisand Guillain-Barré syndrome[28, 104, 120, 144].Theanalysis 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 criteriafor encephalitis[136],with an age distribution curve exhibiting a U-shaped pattern with a very clear trend towards the highest incidenceat the youngest age (Gérardin*et al*., unpublished).In young children, including neonates, neurological complications include encephalitis, seizures and acute encephalopathy.

Mother-to-child transmission of CHIKV infection wasfirst reported during the La Réunion outbreak, as a cause of severe neonatal disease, associated with neurological acute symptoms [39, 40, 46, 103]. The overall prevalence of maternal-fetal transmission is actually low (0.25% after 22 weeks)[39], and vertical transmissionis 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[39]. Whether host and viral genetic factors have an impact on mother-to-child transmission remains to be determined. The preventive role ofCaesarean section on transmission is not precisely known. During the La Reunion outbreak, severe illness was observed in 53% of infected neonatesand mainly consisted ofneurological signs [39]. Importantly,CHIKV infection in the perinatal periodcan cause severe diseasewith lifelong disability, as 51% of infected children exhibit global neurodevelopmental delay[40].

Detailed epidemiological investigations of maternal-fetal CHIKV transmission,as well as human placental and experimental*in vivo*animal studies have led to the conclusionthat vertical contamination most probably occurs as a consequence of passive transfer of maternal bloodfree CHIKV infectiousparticles 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[20, 39]. There is indeed no evidence that CHIKV actively infects and breaches the placental barrier, as for RRV[2, 81].

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[28], 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 rateswere low, approximately1 in 1,000[9, 39, 68, 127].During CHIKV epidemics, a total of 260 excess deaths were reported in La Réunion island (mortality rate attributed to chikungunya 1/1,000)and a total of 2,944 excess deaths occurred in India (Ahmedabad) (mortality rate attributed to chikungunya 0.8/1,000)[56, 78, 79, 98]. Most of the deaths occurred in adults with underlying conditions, as well as, rarely, in neonates.

Chronic chikungunya in human

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[36, 113, 121]. Long-lasting symptomsare typically not observed indengue 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[34] and has been massively reported during all recent epidemics that have occurred since 2005in the Indian Ocean notably in the Island of La Réunion[10, 52, 55, 76, 113, 121, 132]or in metropolitan France for imported cases[23, 66, 119], in India or Southeast Asia[36, 75, 89, 145], in Italy[84]and more recently in the Caribbean[32, 82]. Although the overall proportion of patients with chronic symptoms decreasesover time after CHIKV disease onset (from 100 to 88% during the first 6 weeks, to less than 50% after 3–5 years, with variable findingsdepending 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[36, 113, 121].

Chikungunya chronic disease is characterized by persistent or relapsingarthralgia 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)[36, 55, 113].

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[55]:patients without previously defined arthritis who correspond to 27% of patients,and who present with currentmusculoskeletal disorders (loco-regional or diffuse),and patients withnon-crystalline polyarthritiswho represent 70% of patients fulfilling the diagnostic criteria of rheumatoid arthritis, spondyloarthritis or undifferentiated polyarthritis. The latter were refered toas 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 specializedmedical team is important. Notably, early treatment with anti-inflammatory and immunosuppressive drugssuch as methotrexate might prevent joint damage, although their safety and efficacy remain to be validated in this context[55].

It is noteworthy that joints that are already damaged by underlying disorders, such as osteoarthritis seem to constitute preferential sites for long-term pain[121]. Of note, the likelihood of developing persistent arthralgia is highly dependent on age[55, 113].

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[19]. However, in a study conducted in patients with chronic symptomsup to 36 months after the acute phase, nosystemic biomarkers associated

with chronic arthralgia nor biological markers typically found in autoimmune or rheumatoid diseases were reported[113].

Although the chronic disease generally causesless debilitatingpains than the acute disease, many patients still have a pronounced reduction in movement and quality of life and require long-term treatment[23, 84, 107, 113].

2. Experimental CHIKV infection in animal models mimickingsome featuresof human chikungunya

Animal models for CHIKV infection, including mice and non-human primateshave been used to study CHIKV-associated pathologies[38]. A zebrafish animal model has also been developed and used to visualize CHIKV infection and innate host responses to infection[94]. However, most studies have been conductedin 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 signsfollowing intraperitoneal, intradermal orintravenousvirus inoculations[129].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 inmuscles of the infected foot, leg swelling, edema with evidence of arthritis and tenosynovitis during the acute phase[37, 86].Moreover, newborn and 14-day-old outbread 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[20, 150].They developviremia and skeletal muscle weakness that can be fatal in the case of younger mice and histopathological analysis of the affectedlimb reveals myositis with necrosis.

Adult mice with a complete deficiencyin the type-I interferon receptor ($IFNAR^{-/-}$) develop a severe infection after intra-dermal CHIKV inoculation and toa lesserextentafter

ocular inoculation[20, 22]. 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 copynumber of the type-I IFNreceptorinfluences viral load andtissue distribution, as well as the severity of the disease[20].Therefore, young age and inefficient type-I IFN signalingare 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 monkeywere reported to be susceptible to experimental infection with CHIKVas evidenced by a febrile reaction and high levels of circulating virus[7]. More recently, studies performed in cynomolgus a (*Macaca fascicularis*) and rhesus (*Macaca mulatta*) macaquesexperimentally infected with CHIKV have shown thatinoculation 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)[63, 80]. With the highest infectious dose (10^8PFU), monkeys developed clinical neurological disease characterized by meningo-encephalitis[63]. 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[63].In macaques,CHIKV targets joints, secondary lymphoid organs, liver, and, to a lesser extent, muscle and skin. Interestingly, long-term CHIKV infection can beobserved, mainly in secondary lymphoid organs in cynomolgus macaques[63] and in aged rhesus macaques [80].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 involvespecies-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[58]. Thus, the absence of the proviral effect of NDP52 in the mouse may contribute to itslower 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 alsocontributed to decipher the pathophysiology of the disease (see below).

3. Resolved and pendingquestionsregarding thepathophysiology of CHIKV infection

Whereas the pathophysiology of infection with other alphaviruses, such as SFV and SINVhas been studied for several decades, the pathophysiology of CHIKV infection has been investigated only over the last decade. The studies performedin naturally infected human and experimentally infected animal models have provided cluestothe cell and tissue tropisms of CHIKV,as well ashost factors that control infectionduringthe acute phase of infection.

Cell and tissues tropisms of CHIKV

Acute phase of chikungunya disease

The cell and tissue tropisms of CHIKV at the acute phase of the disease have been investigatedin 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 targetsthe 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[20]. 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. In mouse skeletal muscle, CHIKV canalso be detected, albeit rarely,in satellite cells, consistent with a study performed on human material[92]. 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[48, 85]. Of note, both joint and muscle connective tissues contains 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[21]. 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 muscleand joints, is similar to that of adult IFNAR$^{-/-}$mice, with a pronounced tropism for fibroblasts[20]. 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[150]. Interestingly, in human adult muscle biopsies, myositis together with inflammatory infiltrates mainly consisting of monocytes/macrophages and T cells have been reported[92].Similarly to CHIKV in mouse neonates, RRV has been shown to induce myositis in mice[48, 85, 110].

In the case of severe disease in mice, viremia is high and CHIKV also disseminatesto other tissues, including skin, eye and the central nervous system (CNS). 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[20, 22]. In humans, CHIKV antigens have been found in fibroblasts of the same sites[20, 22]. 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[72, 102].

Together, these data demonstrate that infection of peripheral tissues responsible for symptomsin 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 findingsareconsistent with the*in vitro* observation that human and mouse primary muscle fibroblasts, as well as primary human skin fibroblastsare susceptible to CHIKV infection[20, 29]. Fibroblasts derived from other tissues have also been shown to be permissive to CHIKV, includinghuman lungfibroblasts, primary human foreskin fibroblasts, and mouse embryonic fibroblasts[111, 123]. These cells, as well as primary human skin fibroblasts, produce high level of IFNβtriggered by MAVS activation upon CHIKV infection[29, 111].It has been shown that infection of primary human skin fibroblasts triggers,in addition to IFNβ, enhancement ofIL-1β expression,maturation of caspase-1 and expression of the inflammasome sensor AIM2[29]. Moreover silencing of caspase-1 enhances viral replication. This suggests that CHIKV-infected skin fibroblastsmay contribute to a pro-inflammatory and antiviral microenvironment[29].

The molecular basis for the prominent in vivo tropism for fibroblasts is unknown and may indicate thatfibroblasts could be, relative to other cell types, either (*i*) in ahyper-permissive status regardingCHIKV entry/replication,and/or (*ii*) in a hypo-sensitive status to

type-I IFN-mediatedviral interference, making them a target of choice for CHIKV[20].However, a study has shown that two human fibroblast cell lines with different susceptibility to CHIKV infection failed to showthat differences in the primary type-I IFN and IFN-stimulated genes (ISG)responses toCHIKV were responsible[134]. 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[65].

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[49, 106]. Primary human monocytes and macrophages could also be infected with CHIKV, although with low efficiency[123, 128] and in vivo,viral antigens were detected in monkey macrophages duringthe viremic phase of infection but also at later time points (6 weeks pi)[63], 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 there 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[61]. Another study showed that the murine macrophage cell line Raw264.7 can be infected by CHIKV butthat only a subset of cells is susceptible to infection and produce infectious particles, whereas the others appears to be refractory to infection[62].

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 cellfactors 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[49, 123, 128].

Other in vitro studies have highlighted the specific susceptibility or resistance of cells to CHIKV infection. CHIKV replicates in human muscle satellite cellsbut fails to infect differentiated myotubes[92], 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, suggestingan intracellular block of CHIKV replication in human keratinocytes[6]. According to this finding, the replication of CHIKV in keratinocytes in vivo has thus far never been reported.In vitro, mouse embryonic stem cellsare susceptible to CHIKV infection andsense type I IFNs, butto a lower extent than CHIKV-infected fibroblasts. Moreover, theyare deficient in type I-IFN expression, as compared toCHIKV-infected fibroblasts[138]. 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.

Severe acutechikungunya 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[20, 150]. Primary mouse choroid plexus epithelial cells were highly susceptible to infection, while primary mouse brain microvessel endothelial cells were fully resistant to CHIKV infection[20].In humans,CHIKV and anti-CHIKV IgMs have been detected in the CSF of neonates and adult patients with CNS symptoms[45].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[63]. 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 parenchymalcellsin neonatal/suckling micebut the nature of these cells is unknown[26]. A defective host response may contribute to CHIKV neurotropism in neonates, as well as to the generallyhigher susceptibility of neonates to severe CHIKV infection, in agreement withthe fact that the neonatal immune response is quantitatively and qualitatively distinct from that of adults[3]. In vitro studies show that CHIKV replicates inmouse astrocytes and oligodendrocytes, and with lower efficacy in neuronal cells but fails to infect microglia [25]. 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[31, 147].

Mother-to-child CHIKV transmission has been studied in experimental infection of pregnant animals. 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 andis 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[1, 20, 81].

Chronic phase of chikungunya disease

The pathophysiology of CHIKV chronic disease remainspoorly understood and, to date, no animal model fully reproduces the chronic jointsyndrome associated with many cases. In humans, patients with chronic CHIKV-induced arthralgia often have persistent virus-specific IgM[10, 74], 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[52]. 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[47, 100]. Interestingly, onestudy has demonstrated that viral RNA persists in lymphoid organs and liver and, to a lesser extent, in muscle and joints ofmacaques, and identified macrophages as the main cellular reservoirs during the late stages of CHIKV infection[16, 51, 63], 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 strandedRNA, a product of viral genome replication, is arthrithogenic[148].

In addition to IgM, patients with chronic joint pains can display elevated IL-6 levels[15, 19, 105]. 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[17]. In mouse models, infectious RRV isdetectedin bones and a reduction of bone volumes is observed inassociation with adisruption 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[17]. These findings suggest a mechanism involving direct infection of osteoblasts to explain joint pathologies during infection with RRV and possibly CHIKV[12]. As for RRV, pre-existing arthritis is exacerbated by CHIKV infection[55]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[13, 88,

117].Deciphering the viral and immune mechanisms leading tojoint pathologiesis a health concern, notably in the case of pre-existing arthritis given the relatively high incidence of bone disease in the general population.

Host responseto CHIKV infection

Studies in humans and animal models have shownthat 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.

Innate responseto CHIKV infection

Alphaviruses, including CHIKV, are long known to be strong inducers of type-I IFN and sensitive to type-I IFN responses[30, 41, 43].More recent studies in patients in La Réunion also reported that CHIKV infection elicited high levels ofIFN-α in the serum and its concentration correlatedwith viral load[111, 139].Similar to human patients, the acute disease in monkeys is associated withsubstantially increased type-I IFNconcentration in the plasma[63].As in mammals, CHIKV infection triggers a strong type-I IFN response, critical for survival in CHIKV-infected zebrafish[94].

Recently, the roles of type-I IFNin 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 thisabsence 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, whichleads to antiviral response at the site of injection[111].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-IIFN on nonhematopoietic cells[111], and nonhematopoietic cell-derived type I-IFN is sufficient to control CHIKV infection[112]. 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[108, 111, 112, 143]. 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[112]. 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 includeISG15, ISG20, P56, ZAP, OAS3 andViperin[11, 69, 71, 142, 149].

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[37, 63].Consistent with the role of monocytes/macrophages in inflammation during the acute disease are studies showing that macrophage depletion in mice reducesfoot 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, blocksmonocytes recruitment and reducesjoint inflammation in mice[18, 37, 109]. 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 receptorresultsin more severe disease due to an excessive recruitment of neutrophils rather than monocytes/macrophages to the inflamed joint[99].

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 duringthe 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, showeda 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[57].

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

Adaptive immunityto 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[59, 83, 91, 95]. 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 infectionis also illustrated by the reportthatinfected mice deficient for TLR3 signaling synthesizeanti-CHIKV antibodies that exhibit a lower in vitro neutralization potency than those generated in wt mice[50].

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[47].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[70, 100]. Moreover, theseinfected mice exhibited a more severe disease than wild-type 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[47, 130]. This corroborates observationsmade fromhuman muscle biopsies where T cells, but not B cells, were detected[92].

The importance of B and T cells in protection against CHIKV infections has also been demonstrated by vaccine studies[73]. 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[73, 97].

Finally, the role of hostage 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 [80]. Moreover, while adult animals are able to control viralinfection, 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.

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[8, 77, 114]. In recent years, passive immunization has beeninvestigated 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[21]. 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[4].

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[33, 35]. 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[118]. In other studies, mouse neutralizing antibodies directed against the E1 or E2 protein have been shown to provideboth prophylactic protection from viremia and foot swelling of adult C57BL/6 mice and fromCHIKV-induced lethality in adult

IFNAR$^{-/-}$ mice[42, 93]. Interestingly, combinations of twoneutralizing monoclonal antibody 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 forsevere 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[39]. The protective role of anti-CHIKV hyperimmune human intravenous immunoglobulins in neonates exposed to a high risk of severe form of CHIKVinfection 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.

Conclusions

Repeated, massive CHIKV repeated massive outbreaks of the last decade have elucidatedmany 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 alphaviruses, 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 tobetter prevent and treat the chronic and/or severe diseases caused by CHIKV.

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