Chikungunya fever: Epidemiology, clinical syndrome, pathogenesis and therapy

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Chikungunya virus (CHIKV) is the aetiological agent of the mosquito-borne disease chikungunya fever, a debilitating arthritic disease that, during the past 7 years, has caused immeasurable morbidity and some mortality in humans, including newborn babies, following its emergence and dispersal out of Africa to the Indian Ocean islands and Asia. Since the first reports of its existence in Africa in the 1950s, more than 1500 scientific publications on the different aspects of the disease and its causative agent have been produced. Analysis of these publications shows that, following a number of studies in the 1960s and 1970s, and in the absence of autochthonous cases in developed countries, the interest of the scientific community remained low. However, in 2005 chikungunya fever unexpectedly re-emerged in the form of devastating epidemics in and around the Indian Ocean. These outbreaks were associated with mutations in the viral genome that facilitated the replication of the virus in Aedes albopictus mosquitoes. Since then, nearly 1000 publications on chikungunya fever have been referenced in the PubMed database. This article provides a comprehensive review of chikungunya fever and CHIKV, including clinical data, epidemiological reports, therapeutic aspects and data relating to animal models for in vivo laboratory studies. It includes Supplementary Tables of all WHO outbreak bulletins, ProMED Mail alerts, viral sequences available on GenBank, and PubMed reports of clinical cases and seroprevalence studies.
Chikungunya virus (CHIKV) is an arthropod-borne virus that is transmitted by *Aedes* (*Ae.*) mosquitoes. It was first isolated in 1952 in the Makonde Plateau of the southern province of Tanzania (former Tanganyika). The virus transmission cycle requires infection of female mosquitoes via a viraemic bloodmeal taken from a susceptible vertebrate host and, following a suitable extrinsic incubation period, transmission to another vertebrate host during subsequent feeding (Solignat et al., 2009). After an incubation period, most patients suffer from polyarthralgia and myalgia, with a significant impact on their quality of life. Chikungunya fever is characterised by a very high viraemic load and concomitant abnormalities such as pronounced lymphopenia and moderate thrombocytopenia. The rate of asymptomatic cases is lower, and the percentage of infected patients requiring medical attention is higher, than in most other common arboviral infections. After the acute stage, some patients experienced relapse, persistent arthralgia or musculoskeletal pains. Increase of age is the most obvious risk factor associated with severe disease or persistent symptoms in adults, whilst in paediatric populations, newborns have a higher risk of severe disease.

Since the first reports of chikungunya fever in Africa in the early 1950s, more than 1500 scientific publications on different aspects of the disease and its causative agent have been produced. Analysis of these publications shows that, following a number of studies in the 1960s and 1970s, and in the absence of autochthonous cases in developed countries, the interest of the scientific community remained low (Fig. 1). However, in 2005 chikungunya fever unexpectedly re-emerged in the form of devastating epidemics in and around the Indian Ocean. These outbreaks were associated with mutations in the viral genome that facilitated the replication of the virus in *Aedes albopictus* mosquitoes. Since then, nearly 1000 publications on chikungunya fever have been referenced in the PubMed database. The reader is referred to Supplementary Tables 1–6 for lists of all WHO outbreak bulletins, ProMED Mail alerts, viral sequences available on GenBank, and PubMed reports of clinical cases and seroprevalence studies.

Two distinct transmission cycles have been described for CHIKV: a sylvatic cycle in Africa and an urban human–mosquito–human virus transmission cycle seen in Asia, the Indian Ocean, Africa and more recently, in Europe. The two major vectors of the disease currently identified are *Ae. aegypti* and since 2006, *Ae. albopictus*. The important role of *Ae. albopictus* in recent outbreaks is...
due to adaptive mutations of the viral genome, in particular the A226V mutation in the E1 glycoprotein, that increase viral replication in this specific vector. Based on the partial E1 structural glycoprotein or complete genomic sequences, three phylogroups of CHIKV (West-African, Asian, and East-Central-South-African) which apparently circulate in regions that display different ecological backgrounds have been identified.

No licenced vaccine against chikungunya is commercially available, but several strategies are under study. In sections below we review several drugs which have shown antiviral activity against CHIKV or activity against the inflammatory symptoms associated with CHIKV infection. Treatment of standard presentations of chikungunya fever currently relies on paracetamol/acetaminophen and non-steroidal anti-inflammatory drugs. Chloroquine is not recommended at the acute phase of the disease. Ribavirin has been used in some severe presentations but very limited information is available to confirm its efficacy. Recent investigations may lead to the identification of new antiviral candidates with a clearly defined mechanism of viral inhibition in cell-based systems and significant activity in animal models. Therapeutic protocols for severe cases may also be established based on specific immunoglobulins or molecules that can interfere with some aspects of the inflammatory response associated with CHIKV infection. For chronic rheumatic manifestation and inflammatory polyarthritides lasting more than 2–3 months, disease-modifying anti-rheumatic drugs such as methotrexate are recommended. As described below, studies in animal models suggest that inflammation, macrophage tissue tropism and local viral persistence are involved in the establishment of chronic disease.

2. Chikungunya virus in brief

2.1. Classification

CHIKV belongs to the alphavirus genus of the family Togaviridae (Enserink, 2006; King et al., 2012; Powers and Logue, 2007). CHIKV was first isolated from the serum of a febrile patient during an outbreak that occurred in the southern province of Tanzania (Tanganyika, Makonde Plateau) in 1952–1953 (Robinson, 1955; Ross, 1956). The name “Chikungunya” in the Bantu language of the Makonde people refers to the stooped posture due to the frequent and debilitating joint pain induced during chikungunya fever (Enserink, 2006). There is substantial evidence that CHIKV is transmitted and dispersed by mosquito vectors, in particular by Aedes species (spp.) (Gilotta and Bhattacharya, 1968; Gilotta and Shah, 1967; McIntosh et al., 1963; Paterson and McIntosh, 1964; Sarkar, 1967; Shah et al., 1964; Weinbren et al., 1958).

CHIKV is a member virus of the Semliki Forest (Eurasia) virus antigenic complex, together with a number of other alphaviruses that are found in Africa (O’nyong–nyong virus (ONNV)), in South America (e.g., Mayaro virus) and in the Australia/Oceania region (Ross River virus (RRV)) that cause acute arthropathy in humans (Powers et al., 2000; Vanlandingham et al., 2006).

2.2. Virus structure and genomic organisation

The virion has an icosahedral capsid enclosed by a lipid envelope and a diameter of 60–70 nm. It is sensitive to desiccation and to temperatures >58 °C. The genome is a single-stranded, positive sense, RNA molecule of approximately 12 kb in length ( Khan et al., 2002 ) (Fig. 2). The genomic organisation is arranged as with others alphaviruses: 5′-nsP1-nsP2-nsP3-nsP4-junction region-C-E3–E2–6k–E1-poly (A)-3′ with two open reading frames (ORFs). The 5′ end of the genome has a 7-methylguanosine cap and there is a polyadenylation signal at the 3′ end. The 5′ ORF is translated from genomic RNA and encodes four non-structural proteins (nsP1, 2, 3 and 4) ( Jose et al., 2009 ). The 3′ ORF is translated from a subgenomic 26S RNA and encodes a polyprotein that is processed as the capsid protein (C), two surface envelope glycoproteins (E1 and E2) and two small peptides designated E3 and 6k ( King et al., 2012; Simizu et al., 1984; Voss et al., 2010 ).

The glycoproteins E1 and E2 are embedded in a heterodimeric form in the viral envelope and are responsible for virus attachment and membrane fusion. Virus fusion with the cell membrane is mediated by the E1 glycoprotein, a class II fusion protein, in a process dependent on low-pH. Acidic conditions induce a conformational change in the virus envelope proteins, dissociation of the E2–E1 heterodimers and formation of E1 homotrimers. The E1 trimer is inserted into the target membrane via its hydrophobic fusion peptide and refolds to form a hairpin-like structure. Cholesterol is required for both cell membrane fusion and budding during alphavirus infection ( Solignat et al., 2009 ). A large number of more recent studies have been dedicated to the structural characterisation of the envelope proteins of alphaviruses and to
structural modifications that occur during fusion (Gibbons et al., 2004; Li et al., 2010; Liu and Kielian, 2009; Roussel et al., 2006; Voss et al., 2010; Zhang et al., 2011).

2.3. Replication cycle

The CHIKV replication cycle is essentially similar to the replication cycle of other alphaviruses (Solignat et al., 2009). The non-structural proteins (nsP1–4) and their cleavage intermediates are involved in RNA replication. The five structural proteins (C, E3, E2, 6k, E1) and their cleavage intermediates are required for viral encapsidation and budding (Fros et al., 2010; Leung et al., 2011). Replication occurs in the cytoplasm, both in vertebrate and insect cells, in close association with the Golgi apparatus.

Virus enters cells at the plasma membrane, mostly by endocytosis, via a pH-dependent mechanism, which culminates in fusion pore formation and release of the nucleocapsid into the cytosol. It begins with attachment (E2 is primarily responsible for interactions with cellular receptors) and fusion of virus particles with the host cell membrane. The fusion peptide is located at the tip of the E1 molecule in domain II, close to amino acid 226. Following virus entry, two rounds of translation occur. Positive-sense genomic RNA acts directly as mRNA and is partially translated (5’ end) to produce non-structural proteins (nsP’s). These proteins are responsible for replication and formation of a complementary negative strand, the template for further positive-strand synthesis. Subgenomic mRNA (26S) replication occurs through the synthesis of full-length negative intermediate RNA, which is regulated by nsP4 and p123 precursor in early infection, and later by mature nsPs. Translation of the 26S sub-genomic RNA results in production of 5 structural proteins (C, E3, E2, 6k, E1), which are required for viral encapsidation and budding. Assembly occurs at the cell surface, and the envelope is acquired as the virus buds from the cell. Release and maturation occur almost simultaneously. From Thiboutot et al. (2010), with permission.

3. Clinical syndrome

3.1. Incubation period

The incubation period of chikungunya fever has never been investigated in great detail, but a range of 1–12 days is often cited (Burt et al., 2012; Singh and Unni, 2011). In 1977, an outbreak occurred amongst a group of high-school children from Pretoria after a visit to the northern Transvaal bushveld; two days after their arrival, the first campers were taken ill. Overall, the incubation period during this episode was estimated to vary from 2 to 10 days (Fourie and Morrison, 1979).

Amongst cases imported into metropolitan France during the 2006 Indian Ocean outbreak, 43 patients had a defined onset of disease after their departure from the endemic area. The mean duration of incubation following arrival (which represented the minimal duration of incubation) was 1.6 days (95% CI: 1.1–2.1), with a range of 0–8 days and a median of 1 day (data from Institut de Veille Sanitaire – InVS – 2006, personal communication: Harold Noël). These data are globally in accordance with the medical literature and support the belief that the incubation period is generally short (2–10 days).

3.2. Mild cases and asymptomatic infections

The proportion of individuals infected by CHIKV who develop clinical symptoms requiring medical attention is higher than in most other arboviral infections (Chastel, 2011). Following out-
breaks of chikungunya fever, several recent seroprevalence studies were performed indicating different rates of asymptomatic cases ranging from 3.8% to 27.7%. After the outbreak in Reunion island, 162/967 (16.7%) cases reported no symptoms including 46/967 (4.8%) who ‘did not know’ (Gerardin et al., 2008b). Similarly, after the outbreak in Mayotte, 122/440 (27.7%) cases with positive serology declared no clinical signs that could be compatible with a history of chikungunya fever (Sissoko et al., 2010).

Two years after the chikungunya fever outbreak in the state of Kerala in India, a seroprevalence study found that amongst the 260 patients with positive serology, only 10 (3.8%) declared no symptoms compatible with a history of chikungunya fever (Kumar et al., 2011). In a study amongst French military policemen after the chikungunya outbreak in Reunion island, only 3.2% of cases did not report symptoms (Queyriaux et al., 2008). Finally in a case-control study in Thailand, 9% of laboratory-proven CHIKV infection cases were considered as asymptomatic and the viral loads observed in the symptomatic individuals were not significantly different from those observed in the viremic asymptomatic individuals (Appassakij et al., 2012).

3.3. Clinical features of chikungunya fever

Since the Indian Ocean outbreak in 2005–2006, the information available for the scientific community relating specifically to the clinical characteristics of patients infected by CHIKV has significantly increased. Two stages of the disease are now described: acute illness and the late stage of illness, with persistent arthropathy.

3.3.1. Acute illness

Several recent and prospective studies are currently available to describe accurately the acute stage of illness (Randyopadhyay and Ghosh, 2010; Borgherini et al., 2007; Chow et al., 2011; Nkoghe et al., 2012; Queyriaux et al., 2008; Rezza et al., 2007; Riyaz et al., 2010; Simon et al., 2007; Staikowsky et al., 2009; Taubitz et al., 2007; Thiberville et al., 2013; Win et al., 2010).

Symptomatic patients generally report an abrupt onset of disease characterised by high fever, polyarthralgia, backache, headache, and fatigue. Fever and characteristic clinical symptoms appear within 4–7 days.

Polyarthralgia is reported in 87–98% of cases and represents the most characteristic symptom. Joint pain is mostly polyarticular, bilateral, symmetrical and occurs mainly in peripheral joints (wrists, ankles, and phalanges) and some large joints (shoulders, elbows and knees). Joint swelling is less frequent: 25–42% of cases. Pains in the ligaments (pubalgia, sternocleidomastoid, occipital insertions and talalagia), temporo mandibular and sternocostoclavicular joints and tenosynovitis, have also been described. Myalgia was observed in 46–59% of cases in recent prospective studies, whilst retrospective studies reported a higher prevalence (93%). Myalgia has been observed predominantly in the arms, thighs and calves without myositis.

Cutaneous manifestations were reported in 40–50% of cases and characterised by a macular or maculopapular rash involving mainly the extremities, trunk and face. The skin lesions are transient and mostly occur a few (2–5) days after the onset of the disease. Generalised pruritus was reported in one-fourth of cases. A large variety of skin and mucous membrane lesions has also been reported during the acute stage of the disease: hypermelanosis, hyperpigmentation, photosensitivity, exfoliative dermatitis, vesicles, bullae, vasculitic lesions, erythema nodosum like lesions, exacerbation of pre-existing dermatoses such as psoriasis and mucosal ulceration.

Digestive symptoms such as diarrhoea, vomiting, nausea or abdominal pain occurs in 15–47% of cases during the acute stage of the disease. As a consequence, chikungunya fever has a major impact on quality of life during the acute stage of illness. Incapacitation or limitation of normal activity occurs in more than 60% of cases whilst tiredness is considered as significant or very significant in 47% of cases. Psychological impact was observed and some patients were depressed or demoralised.

More recently, a study described the classical and daily evolution (until day 14 after onset) of the main symptoms of a cohort of outpatients infected by CHIKV during the Reunion island outbreak. Two stages were identified: the ‘viral stage’ (days 1–4), associated with rapid decrease of viraemia, followed by rapid improvement of clinical presentation and the ‘convalescent stage’ (days 5–14) that was associated with no detectable viraemia and a slow clinical improvement (Thiberville et al., 2013).

3.3.2. Viraemia and changes in clinical laboratory tests

The acute stage of chikungunya fever is characterised by a very high viraemic load (10^6 pfu/ml on average) with a median duration of viraemia of 6 days (range 3–10 days) and concomitant abnormalities such as pronounced lymphopenia and/or moderate thrombocytopenia. In a prospective series of cases during the Reunion island outbreak in 2006, 79% of patients had a lymphopenia (<1000 cells/mm^3), which was severe (<500 cells/mm^3) in 39% of cases. Lymphopenia was more pronounced in patients with higher viraemia. Moderate thrombocytopenia (<150,000 cells/mm^3) and >100,000 cells/mm^3) was recorded in 40–50% of patients. Other less common biological abnormalities have been observed such as leukopenia, elevated liver enzymes, anemia, elevated creatinine, elevated creatinine kinase and hypocalcemia.

3.3.3. Differential diagnosis

Differentiating chikungunya fever from other possible etiologies is important for physicians, notably to ensure good management of outpatients. In a retrospective serological study in Mayotte Island after the outbreak of 2006, Sissoko et al. (2010) found that fever together with polyarthralgia provided an 84% sensitivity (Se) with a 74% positive predictive value (PPV) and an 83% negative predictive value (NPV). In a prospective study of patients with suspected chikungunya fever during a recent outbreak in Gabon, fever and arthralgia had a Se of 73% with a PPV and NPV of 79% and 44% respectively (Nkoghe et al., 2012). In another study, Staikowsky et al. (2009) reported that CHIKV positive patients had an increased frequency of skin rashes, arthralgia on the feet and wrist, asthenia, a higher temperature and a lower frequency of digestive symptoms and pruritus than CHIKV negative patients.

In a retrospective study aiming to compare dengue and chikungunya fever on presentation, chikungunya was independently associated with arthralgia and rash, whilst dengue was associated with myalgia, raised aspartate transaminase, and leucopenia (Mohd Zim et al., 2013). Biological parameters had also been associated with CHIKV-positive patients, notably lymphopenia, elevated liver enzymes, anemia, and elevated creatinine (Nkoghe et al., 2012; Staikowsky et al., 2009). More recently, clinical and clinico-biological diagnostic scores based on a cohort of outpatients during the Reunion island outbreak have been proposed, using very simple criteria (arthralgia on hands and wrists, minor or absent myalgia that can be combined (clinico-biological score) or not (clinical score) with the presence of lymphopenia (<1G/L)) (Thiberville et al., 2013).

3.3.4. Late stage of illness and persistent arthropathy

After the acute stage described above, some patients experience relapse or persistent symptoms. The clinical description and the risk factors of the late stage have recently gained increased interest
as evidence by several studies (Borgherini et al., 2008; Bouquillard and Combe, 2009; Couturier et al., 2012; de Andrade et al., 2010; Gerardin et al., 2011; Hoaraü et al., 2010a; Manimunda et al., 2010; Marimououtou et al., 2012; Moro et al., 2012; Narasimulu and Prabh, 2011; Nkoghe et al., 2012; Ribera et al., 2012; Simon et al., 2007; Sissoko et al., 2009; Thiberville et al., 2013; Win et al., 2010).

Amongst the symptoms declared during the late stage, arthralgia or musculoskeletal pains were the most frequent long-lasting signs. Following the chikungunya fever outbreak in Reunion island, several studies have prospectively followed-up patients who presented with chronic rheumatic manifestations. Rheumatoid arthritis (RA) according to the American College of Rheumatology (ACR) criteria, spondyloarthropathy and other non-classified rheumatism were diagnosed and associated with the chikungunya fever. Others symptoms were less frequently reported such as fever, fatigue, headaches, neuropathic pain syndromes, cerebral disorders, sensorineural impairments, dysesthesia and/or paraesthesia, carpal, tarsal, or cubital tunnel syndromes, digestive disorders, skin involvement, rash, alopecia, pruritus, bilateral Raynaud phenomenon, or erythegalma, joint stiffness, bursitis, tenosynovitis and synovitis with or without effusions. As a consequence, the quality of life and psychological health could be impaired at the late stage. Except for some patients with a diagnosis of RA, standard laboratory findings including inflammatory markers are remarkably within normal limits during this stage.

The proportion of chikungunya patients who fully recovered, partially improved or had persistent symptoms vary between studies and according to the time of the study assessment from the onset of the disease. In all cases, the frequency of persons presenting with symptoms of chikungunya fever decreased with increasing time of onset. In a cross-sectional study after the outbreak on Reunion island, 16% of patients declared that they had suffered for less than one month, 31% between one and three months and 53% still suffered, on average, 128 days after the onset of the disease.

In an outpatient cohort during the Reunion island outbreak, approximately 20% of patients declared incomplete recovery or persistent arthralgia 300 days after the onset of the disease. Twelve months after the outbreak in Italy, 66% and 60% of cases still reported at least one of the most frequent symptoms (arthralgia, myalgia and asthenia) or only arthralgia respectively. In another study, 15 months after the outbreak of Mayotte 2006, 36% of patients reported that they had persistent symptoms and 21% reported experiencing at least one episode of recurrence. In contrast, during the outbreak of Gabon 2010, 83% of patients recovered fully by 30 days after the onset of the disease. In West India after the 2006 epidemic, 12% and 5% suffered from musculoskeletal pains and arthrits at 1 and 2 years after the disease onset respectively.

3.3.5. Risk factors for persistent arthropathy

Dupuis-Maguiraga et al. (2012) have reviewed the host and virological factors recorded during the late stage of clinical disease. Increased age was associated with a longer duration of illness and the presence of joint pain at the late stages of infection. In contrast age was not a risk factor for chronic disease during the outbreak in Singapore, possibly due to a younger population being studied. Pre-existing joint pain or osteoarthritis comorbidity are also associated with an increased risk of persistent symptoms. Female gender was associated with a longer duration of illness and persistent symptoms, but only after univariate analysis.

Amongst the biological markers of the acute stage, viral load has been associated with persistent symptoms during the Reunion island outbreak whereas no relationship between viral load and late stage was found during the outbreak in Singapore. A strong early Immunoglobulin G3 (IgG3) response triggered by a high viral load was also proposed to protect against chronic long-term effects of CHIKV infection. Recently, two studies have observed a positive association between high titres of CHIKV specific IgG antibodies and long lasting arthralgias (Gerardin et al., 2013; Moro et al., 2012). In one study, peak creatinine levels were significantly lower in patients with persistent arthralgia (Win et al., 2010).

3.4. Atypical cases

Since the 1960–1970s CHIKV has been known to affect the central nervous system. During the recent Indian Ocean outbreak, neurological complications were reported in less than 25% of cases. Seizures were most often reported in patients who had a past medical history of epilepsy and/or in those with a history of heavy alcohol consumption. Other neurological complications reported are encephalopathy, encephalitis, Guillain–Barre, encephalomyeloradiculitis or subarachnoid cerebellar haemorrhages (Borgherini et al., 2007; Das et al., 2010; Staikowsky et al., 2009).

Haemorrhagic signs are rare (1–7% of patients) and minor, such as bleeding of the nose and gums (Borgherini et al., 2007; Win et al., 2010). In most cases, they are not associated with clotting abnormalities or major thrombocytopenia (Nkoghe et al., 2012; Staikowsky et al., 2009).

A variety of other clinical symptoms have been reported during the acute stage of chikungunya fever, such as conjunctivitis, neuroretinitis, iridocyclitis, myocarditis, pericarditis, pneumonia, dry cough, lymphadenopathy, nephritis, hepatitis and pancreatitis (Borgherini et al., 2007; Economopoulou et al., 2009; Mahendradas et al., 2008; Mirabel et al., 2007; Nair et al., 2012; Rajapakse et al., 2010; Renault et al., 2007; Rezza et al., 2007; Rose et al., 2011; Simon et al., 2007; Simon et al., 2008; Staikowsky et al., 2009; Win et al., 2010). Overall, atypical cases were estimated at 0.3% of all symptomatic cases during the 2006 Reunion island outbreak (Economopoulou et al., 2009). Amongst these atypical cases, 36% were considered to be severe, 14% were admitted to an intensive care unit and 10% died (Economopoulou et al., 2009). The reported causes of death were heart failure, multiple organ failure syndrome, toxic hepatitis, encephalitis, biliary dermatosis, respiratory failure, renal failure, pneumonia, acute myocardial infarction, cerebrovascular disease, hypothyroidism or sepsicaemia.

During the Reunion island outbreak, an excessive death-rate was observed and mortality associated with chikungunya fever was reported to have had a case-fatality ratio of about 1 in 1000 (Josseran et al., 2006). A similar case-fatality ratio was observed following the outbreak in Port Blair, capital city of the Union Territory of Andaman and Nicobar Islands, India, in 2006 (Manimunda et al., 2011). However, the comparison between expected and observed mortality in Reunion island, 2006, identified a high-mortality rate between January and May and a low-mortality rate during the rest of the year (Renault et al., 2012), suggesting the existence of a ‘harvesting’ phenomenon and implying that the global excess of death might have been overestimated when derived only from the epidemic period.

3.5. Chikungunya fever in children

In children, clinical manifestations of chikungunya fever appear to be quite specific and although rheumatological manifestations are less frequent, they remain a group at high risk of atypical or severe manifestations. The main clinical characteristics in infants are the high prevalence of dermatological manifestations (hyperpigmentation, generalised erythema, maculopapular rash and vesiculobullous lesions) and neurological complications (encephalitis, seizures, meningeval syndrome or acute encephalopathy). Other clinical features are also described such as digestive disorder (loose stools), peripheral cyanosis and minor haemorrhagic
manifestations (Robin et al., 2008, 2010; Sebastian et al., 2009; Valamparampil et al., 2009).

3.6. Chikungunya fever in pregnant women

Although chikungunya fever apparently has no observable teratogenic effects during pregnancy (Fritel et al., 2010), vertical transmission was reported for the first time, during the 2006 Reunion island outbreak and was observed exclusively in near-term deliveries in the context of intrapartum viremia, with a rate of 49%. Cesarean section had no protective effect on transmission. Severe illness was observed in 53% of newborns and mainly consisted of encephalopathy with persistent disabilities in 44% of them (Gerardin et al., 2008a). The others complications included seizures, haemorrhagic syndrome, haemodynamic disorders, cardiologic complication (myocardial hypertrophy, ventricular dysfunction, pericarditis, coronary artery dilatation), necrotizing enterocolitis or dermatologic manifestation (Nair, 2008; Ramful et al., 2007; Rao et al., 2008).

3.7. Risk factors for severe disease

Risk factors for severe acute disease have been thoroughly investigated (Boherinri et al., 2007; Chow et al., 2011; Economo-poulou et al., 2009; Gerardin et al., 2008a; Gerardin et al., 2008b; Kam et al., 2012c; Kee et al., 2010; Kumar et al., 2010; Loh-achanakul et al., 2012; Ng et al., 2009a; Nkoghe et al., 2012; Paquet et al., 2006; Renault et al., 2007; Sisoko et al., 2008; Staikowsky et al., 2009). Several studies reported that, in adults, the incidence of atypical cases, severe cases, hospitalisation and the mortality rate increased with age. In pediatric populations, newborns had a high risk of severe disease. Although comorbidity and increase of age are often linked, comorbidity or underlying respiratory diseases, the use of NSAIDs prior to hospitalisation, hypertension and underlying cardiac disorders have been associated with hospitalisation or disease severity. Alcohol abuse was also associated with increased mortality.

Female gender has not been clearly associated with more severe illness but, during the Indian Ocean outbreaks, women were over-represented based on reported cases, whereas cross-sectional studies found similar seroprevalence values for both genders, suggesting a variability of symptomatic disease depending on gender. In contrast, in a single study, males and blood Rhesus-positive individuals were found to be more susceptible to infection by CHIKV. Case reports of immunocompromised patients have described atypical and severe disease but no studies have compared immunocompromised or immunodeficient patients. The association between viral load and acute severe illness is controversial. Indeed, several studies reported higher viral load in hospitalised patients or patients with severe illness whilst others studies did not reveal significant association between the viral load and the clinical presentation. Other biological abnormalities or immunological markers have been proposed to be associated with disease severity such as elevated liver enzyme, creatinine, C reactive protein (CRP), hypocalemia, elevated IL-1β or IL-6, decreases in RANTES or early CHIKV-specific IgG3.

3.8. Supportive therapy

No studies have precisely evaluated the efficacy of the various symptomatic treatments usually employed during the acute or chronic stage of chikungunya fever. Nevertheless, in previously published reviews, paracetamol/acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) have been recommended. Aspirin should be avoided because of the risk of bleeding. Systemic corticosteroids should also be avoided because of the strong rebound effect when the treatment is stopped (Ali Ou Alla and Combe, 2011; Burt et al., 2012; Simon et al., 2011; Singh and Unni, 2011).

For severe chronic arthritis, disease-modifying anti-rheumatic drugs (DMARDs), including methotrexate, hydroxychloroquine or sulphasalazine have been proposed (Bouquillard and Combe, 2009). Although anti-tumour necrosis factor-α (anti-TNF) has been associated with an exacerbated disease in mouse models at the early stage of illness (Zaid et al., 2011), patients with a diagnosis of post-chikungunya rheumatoid arthritis have been successfully treated with anti-TNF antibodies (Bouquillard and Combe, 2009).

Ribera et al. (2012) recently proposed a therapeutic protocol for patients with chronic rheumatic manifestation; for patients without a diagnosis of polyarthritis symptomatic treatment such as analgesic ladder 1 (paracetamol, NSAIDs) or 2 (codeine, tramadol) with kinesitherapia, muscle relaxant drugs, acupuncture and physiotherapy; for patients with a defined inflammatory polyarthritis during more than 2–3 months, DMARDs (methotrexate) are proposed. For patients who do not respond well to the usual analgesics at the chronic stage, neuropathic syndromes should be more carefully evaluated to assess whether or not specific treatments such as antiepileptics and antidepressants could be used to alleviate suffering (de Andrade et al., 2010).

4. Epidemiology

4.1. Arthropod vectors

The main CHIKV mosquito vectors are Ae. aegypti (Stegomyia aegypti) and Ae. albopictus (Stegomyia albopicta). Until recently, Ae. aegypti was considered the primary vector for CHIKV transmission but in 2006, Ae. albopictus was surprisingly identified as a second major vector of the virus both in places where Ae. aegypti is considered to be rare (e.g., in Reunion island) and also in places where both mosquito species are prevalent (e.g., in Madagascar, India and Gabon). This emergence of Ae. albopictus as a major vector of CHIKV was largely attributable to a single mutation in the E1 protein of CHIKV which facilitated enhanced virus uptake, replication and transmission by the vector (Tsatsarkin et al., 2011b).

Ae. albopictus has recently shown a remarkable capacity to adapt to peri-domestic environments, enabling it to displace Ae. aegypti in some places and to become a significant vector of CHIKV and DENV (Knudsen, 1995). In Africa, a wide range of other mosquitoes has also been incriminated in the transmission of CHIKV, such as other Ae. spp. (Ae. furcifer, Ae. taylori, Ae. vittatus, Ae. fulgens, Ae. luteocephalus, Ae. dalzieli, Ae. vigilax, Ae. camptorhynites, Ae. africanus) and also Culx annullirostris and Mansonia uniformis (Lam et al., 2001; Yadav et al., 2003).

4.2. Vertebrate reservoirs and transmission cycle

Human beings serve as reservoir hosts for the virus during epidemic periods whereas during inter-epidemic periods, several other reservoir hosts have been incriminated such as monkeys, rodents and birds (Inoue et al., 2003; Wolfe et al., 2001). The significance of non-human primates as reservoir hosts is discussed in the section devoted to pathogenesis.

Two distinct transmission cycles have been described for CHIKV: a sylvatic cycle in Africa (Wolfe et al., 2001), the general pattern of which resembles the yellow fever virus sylvatic cycle (i.e., involving virus transmission between forest—or sylvan—associated mosquitoes and non-human primates (and possibly rodents) with occasional spillover of the virus into nearby human populations living in or close to the sylvatic environment). Under these latter circumstances the virus may then encounter mosquitoes primarily associated with urban environments (i.e., domestic
or peridomestic mosquitoes) thus initiating an urban human–mosquito–human virus transmission cycle such as those seen in Asia, the Indian Ocean, Africa and more recently, Europe (Rezza et al., 2007). In general, when *Ae. aegypti* is the predominant vector, these urban outbreaks or epidemics resemble the virus transmission cycle of dengue virus in the urban environment.

In Africa, CHIKV is maintained in a sylvatic cycle involving wild non-human primates and a variety of forest-dwelling mosquito species (Powers and Logue, 2007), such as *Ae. africanus* in Uganda and Bangui, *Ae. cordeliier* in South Africa, *Ae. furcifer–taylori* in South Africa and Senegal (Diallo et al., 1999) and *Ae. luteocephalus* and *Ae. dalzieli* in Senegal (Diallo et al., 1999; Jupp and McIntosh, 1990; McIntosh et al., 1963). In rural regions of Africa, the outbreaks tend to affect small populations and appear to be heavily dependent on the sylvatic mosquito population densities that increase during periods of heavy rainfall (Diallo et al., 1999; Thonnon et al., 1999). However, during recent outbreaks in Cameroon, Congo and Gabon, CHIKV was vectored by the Asian mosquito *Ae. albopictus* which has gradually widened its geographic range, out of Asia into Africa, Europe and the Americas (Benedict et al., 2007). Currently, the principle factors (if indeed there are any) responsible for the maintenance of this *Ae. albopictus*-adapted virus life cycle in the sylvatic environment have not been defined.

In Asia, CHIKV is maintained in human-mosquito-human urban cycles involving both *Ae. aegypti* and *Ae. albopictus*. Whether or not the transmission cycles are exclusive for each mosquito species is not yet known and has not yet been fully investigated. For many years, CHIKV persistence was thought to depend primarily upon continuous introductions of the virus into immunologically naïve populations (Powers and Logue, 2007). However, the evidence for long lasting persistence of the Asian genotype (see infra) and several recent studies suggest that sylvatic cycles could also play a part in the transmission of CHIKV in Asia (Powers et al., 2000; Apandi et al., 2009).

### 4.3. Infection in humans

CHIKV is endemic and epidemic in Africa, Asia and since 2005, the Indian Ocean (Kariuki Njenga et al., 2008; Powers and Logue, 2007). Cases of chikungunya fever, retrieved from the literature and from different public health alert systems, are shown in Fig. 4A and B. The following paragraphs summarise the information relating to virus circulation in original endemic areas (i.e., Africa and Asia), to the recent epidemiological expansion and to the possible introduction of the virus in non-endemic areas by infected travellers (Fig. 5). Supplementary Tables 1–6 list all WHO outbreak bulletins, ProMED Mail alerts, viral sequences available on GenBank, and PubMed reports of clinical cases and seroprevalence studies.

#### 4.3.1. Early description of chikungunya

The first human documented epidemic caused by CHIKV was recognised in East Africa and Austral Africa (Tanzania, Uganda and Zimbabwe) in 1952 and 1953 (Mason and Haddow, 1957; Weinbren, 1958; Weinbren et al., 1958). In Asia, the first epidemic was documented in 1958 in Bangkok (Aikat et al., 1964; Volk et al., 2010), followed by a number of outbreaks documented in the Philippines, Cambodia, Vietnam, Laos, Myanmar, Malaysia and Indonesia (Halstead et al., 1969a,b).

Between 1954 and 1990, the virus was implicated as the cause of epidemics in:

- The Philippines, Thailand, Myanmar (Khai Ming et al., 1974),
- Singapore, Sri Lanka (Hermon, 1967; Mendis, 1967; Vesenjak-Hirjan et al., 1969),
Other ancient possible cases of chikungunya fever in Asia have been reported (Carey, 1971; Ng and Hapuarachchi, 2010) that include a widespread epidemic of self-limited febrile illness in Africa, the Caribbean, West Indies and India in the 1820s, with a large proportion of the population of Calcutta (current Kolkatta, India) affected, and subsequent cases in Calcutta in 1853 and 1871, possibly originating from Zanzibar and then spreading to Aden (Red sea), Calcutta, Burma (Myanmar) and Java. Of note, whilst this epidemic pattern would locate the most ancient reported cases of chikungunya fever in Asia, it is fully compatible with an African origin of the outbreaks.

4.3.2. CHIKV epidemics in Africa and Asia

4.3.2.1. In Africa. Since 1960, cases of chikungunya fever have been reported in several African countries (see Supplementary Table 6):

- South Africa (McIntosh et al., 1963; Paterson and McIntosh, 1964; Powers and Logue, 2007)
- Angola (Filipe and Pinto, 1973; Pinto and Filipe, 1973)
- Niger and Nigeria (Moore et al., 1974; Powers and Logue, 2007; Tomori et al., 1975)
- Central African Republic (Pastorino et al., 2004; Saluzzo et al., 1980)
- Democratic Republic of Congo (Pastorino et al., 2004)
- Ivory Coast, Malawi and Sudan (Watts et al., 1994)
- Senegal (Diallo et al., 1999; Powers and Logue, 2007; Roche and Robin, 1967; Thonnon et al., 1999)
- Zimbabwe (Powers and Logue, 2007)
- Uganda (Powers and Logue, 2007)
- Kenya (Kariuki Njenga et al., 2008; Powers and Logue, 2007)

The Democratic Republic of Congo declared an epidemic in 1999–2000 (Muyembe-Tamfum et al., 2003). During the period 2004 through 2010, epidemics were identified sequentially in Kenya, Senegal, Sudan (2005), the Cameroon (2006) and Gabon (2007 and 2010). In 2011, an epidemic was reported for the first time in the Republic of Congo (Kelvin, 2011).

Paradoxically, our knowledge of the natural history and environmental cycle of the virus in Africa remains fragmentary. Whilst infection of nonhuman primates (which may act as reservoir or amplifying hosts) has been demonstrated (see section below on pathogenesis), and the virus identified in a variety of sylvatic mosquitoes (see previous sections) the determinants or emergence remain poorly understood. In both Africa and Asia, outbreaks were reported to be unpredictable, with an interval of 7–20 years between individual epidemics (Powers and Logue, 2007) and an interval of 4–5 decades between major epidemics. Whilst the precise factors underlying this pattern are unknown, it is probable that, in addition to a variety of ecological and viral genetic factors and given the long lasting immune protection acquired after chikungunya infection, the immune status of the populations plays a central role in the periodicity of recurrence. This is in coherence with the observation that recurrent outbreaks preferentially target the youngest age-groups (i.e., non immunised during the previous epidemics) (Robert et al., 1996).

4.3.2.2. In Asia. More recent epidemic re-emergence was documented in 2001–2003 in Java (Indonesia), after a 20-year period of its absence (Laras et al., 2005)(see next section). The first ever case of CHIKV in the Hong Kong Special Administrative Region was reported on 24 March 2006 (corresponding to an imported
case from Mauritius (ProMED-mail accession number 20060402.0989)). In 2010, an outbreak in Guangdong province (China) of chikungunya fever was reported (Wu et al., 2012). Interestingly, a detailed clinical report by David Bylon in the city of Batavia (current Jakarta, Indonesia) in the year 1779 is highly evocative of chikungunya (see Ng and Hapuarachchi, 2010) and may have been for a long time mistakenly reported as a documentation of dengue fever (Carey, 1971; Halstead, 2009).

Our understanding of the origin of the Asian genotype of CHIKV remains fragmentary. It is probable that the first documented case reported in Bangkok in 1958 reflected more ancient circulation of CHIKV in the region, thus explaining the extent of genetic divergence between the East-Central-South-African (ECSA) and Asian genotypes. According to Ng et al. (Ng and Hapuarachchi, 2010) the availability of historical clinical records suggestive of chikungunya fever outbreaks in Asia since 1779 suggests the independent evolution of an African ancestor of CHIKV in Asia for several centuries, resulting in the Asian genotype. However, this is difficult to accommodate with the most recent estimates of CHIKV evolutionary rates since the most recent ancestor of the Asian and ECSA genotypes is placed at the beginning of the 20th century (Cherian et al., 2009; Volk et al., 2010). Alternately if the report of chikungunya fever in Jakarta in 1779 is exact, it may implicate a limited importation of CHIKV from Africa, the installation of the Asian genotype occurring later (possibly at the end of the 19th century).

Globally, the chikungunya epidemiological situation in Asia remains poorly documented. It is probable that the first documented case reported in Bangkok in 1958 reflected more ancient circulation of CHIKV in the region, thus explaining the extent of genetic divergence between the East-Central-South-African (ECSA) and Asian genotypes. According to Ng et al. (Ng and Hapuarachchi, 2010) the availability of historical clinical records suggestive of chikungunya fever outbreaks in Asia since 1779 suggests the independent evolution of an African ancestor of CHIKV in Asia for several centuries, resulting in the Asian genotype. However, this is difficult to accommodate with the most recent estimates of CHIKV evolutionary rates since the most recent ancestor of the Asian and ECSA genotypes is placed at the beginning of the 20th century (Cherian et al., 2009; Volk et al., 2010). Alternately if the report of chikungunya fever in Jakarta in 1779 is exact, it may implicate a limited importation of CHIKV from Africa, the installation of the Asian genotype occurring later (possibly at the end of the 19th century).

Globally, the chikungunya epidemiological situation in Asia remains poorly documented. First, the respective roles of the endemic Asian genotype and of the other genotypes (including the viruses responsible for the Indian Ocean outbreak) have been poorly investigated, despite evidence for co-circulation in some areas (e.g., India, Indonesia. . . see Fig. 6). As noted (Ng and Hapuarachchi, 2010), whilst the ECSA genotype has been taking centre stage in the recent Asian outbreaks, the Asian lineage has not faded into oblivion, with recent cases reported in Malaysia (Kumarasamy et al., 2006), Singapore (Ng and Hapuarachchi, 2010) and Taiwan (Huang et al., 2009) (see also Fig. 6). The long-standing persistence of the Asian genotype, and the recent isolation of an Asian genotype strain from wild macaque monkeys in Malaysia (Apani et al., 2009) are evocative of a sylvatic maintenance which remains to be further documented (Powers et al., 2000). Secondly, seroepidemiological studies are required to understand in more depth, the level of exposure of the populations and thus to propose a cartography of risk.

4.3.3. Epidemics in the Indian Ocean, India and Southeast Asia

In May 2004, an outbreak of chikungunya fever affected the population on the Kenyan coast. It was first identified on Lamu island where the epidemic peak was reached in July (Renault et al., 2012). It subsequently appeared on the Comoros Islands in December 2004 (Paquet et al., 2006; Schuffenecker et al., 2006) where the epidemic peak was recorded at the end of March 2005, mostly affecting Grande Comore (Ngazidja) island. The epidemic then encompassed the inhabited islands in the entire Southwest Indian Ocean (Mayotte, Reunion Island, Mauritius, Seychelles and Madagascar) (Fig. 5).

Importantly, this outbreak started and spread in areas where the most prevalent vector is *Ae. aegypti* (i.e., Kenya, Comoros, Seychelles, some areas of Madagascar). Transmission by this mosquito was extremely efficient since a cross-sectional seroprevalence study, performed 9 weeks after the epidemic peak on a representative sample of the Lamu island (Kenya) population, revealed that 75% of the population presented with anti-chikungunya antibodies (Renault et al., 2012; Sergon et al., 2007).

![Fig. 5. Dispersal pattern of CHIKV from Africa to the Indian Ocean and Europe during the past 20–50 years. Viral evolution and spread are represented according to recent phylogenetic studies. Different evolutionary lineages are identified using arrows with specific colours. This figure was reproduced with permission (de Lamballerie et al., 2008).](image-url)
Little information is available regarding the situation in Mauritius (Renault et al., 2012), where the most prevalent vector is *Ae. albopictus*. The first detected epidemic wave occurred in March–June 2005, with a second peak in March 2006 (Laras et al., 2005). No case has been reported since August 2006. In the absence of seroprevalence data, the number of cases officially declared (less than 1% of the population) is presumably considerably underestimated.

In the Seychelles archipelago, the first suspected case was reported in July 2005, with a first limited (11 cases) peak in September 2005. The second peak occurred in February 2006 and (probably reflecting the limited impact of the first waves) a third peak occurred in May–June 2007. The total attack rate was estimated to be 12% of the population. Since the end of December 2007, a few suspected cases have been occasionally notified. No retrospective seroprevalence study was conducted.

In Madagascar, epidemiological data are scarce, with initial cases mostly established in travellers returning from the island and clinical epidemiological studies made difficult by the co-circulation of dengue during the same period (Renault et al., 2012). The impact of the 2006 chikungunya outbreak was probably important, and chikungunya fever is suspected to be now endemically present in the island since several cases imported from Madagascar have been identified in Reunion island between 2009 and 2010. It is probable that an outbreak affected the South-east of Madagascar in 2010, which was considered over at the end of March 2010. No seroprevalence data are available.

In Reunion and Mayotte islands, which are French overseas territories, the 2006 chikungunya outbreak was analysed in detail (Charrel et al., 2007; Pialoux et al., 2007; Renault et al., 2012). On Reunion island, where *Ae. albopictus* is the only epidemiologically relevant vector, the first peak of the outbreak was observed in May 2005 (450 cases). The incidence then decreased and became stable at around 100 cases per week during the austral winter (Renault et al., 2012). A second peak was reached in January–February 2006 (with up to 47,000 cases in a single week in a population of less than 800,000 inhabitants). The last indigenous case was reported in December 2006.

A retrospective seroepidemiological study showed that approximately 40% of the population had been infected, demonstrating the massive impact of the epidemics caused by this dispersing virus (Gerardin et al., 2008b). This is in agreement with prospective surveillance data which reported 266,000 symptomatic infections (attack rate: 34%) and 22 severe presentations requiring assistance for at least one vital function (Renault et al., 2012), of whom 65 died (29%); 25 severe presentations in patients under 15 years of age were detected, of whom 2 died (8%). Forty four cases of mother-to-infant transmission were reported (Economopoulou et al., 2009).

In 2009, a new indigenous cluster was observed in the West of the island (5 cases, D’Ortenzio et al., 2009) and in 2010 a second cluster was detected in the same region in March (Saint-Paul, more than 150 cases, first case detected on March 17). Epidemiological investigations established that the probability of unnoticeable virus circulation on the island was unlikely and that the most probable origin of the outbreak was imported cases from Madagascar associated with high vector density. During the outbreak, the house index and Breteau index collected around chikungunya fever cases was much higher than the level of epidemic risk defined by WHO and comparable with levels observed during the 2005–2006 outbreak (D’Ortenzio et al., 2011; Vilain et al., 2012). This result demonstrates that a global level of immunity around 40% was
not sufficient to prevent autochthonous transmission of chikungunya fever on Reunion island.

In Mayotte, 63 cases were detected in February–June 2005. A second peak was observed in 2006, with an attack rate estimated at 4% (Renault et al., 2012). No case has been notified since April 2006. In Sri Lanka and the Maldives, the outbreaks appeared in November and December 2006 respectively (WHO, 2007; Yoosuf et al., 2009), followed by explosive epidemics, involving millions of people in at least 13 Indian states, (Kalanti et al., 2006; Lahariya and Pradhan, 2006) where the virus had apparently been virtually absent for the previous 32 years (it is not widely reported that cities in India had experienced massive outbreaks of chikungunya fever during the 1960s and early 1970s). The recent epidemics then spread to Pakistan, Malaysia, Singapore and Thailand (Pulmanauasahakul et al., 2011).

4.3.4. Chikungunya fever in Europe and the Americas

4.3.4.1. Imported cases. Imported cases have been reported in France (Cordel et al., 2006; Grandadam et al., 2011; Krastinova et al., 2006), Germany (Frank et al., 2011), Switzerland (Bodennmann and Genton, 2006) and Norway (WHO, 2006) and, based on public health reports, in other countries such as the United Kingdom, Belgium, Spain and the Czech Republic (see Supplementary Tables). Surprisingly, more than 1000 imported cases were reported in 2006 in Western Europe. An attempt at creating risk stratified surveillance zones in Europe indicated that France and Italy are likely to be at greater risk due to the number of visitors they receive from chikungunya active regions, principally viremic visitors from India (Tilston et al., 2009).

Between 2006 and 2011, imported cases were also reported in North America, Canada, French Guyana, Brazil, Guadeloupe, Martinique, New Caledonia and Australia (Bonilauri et al., 2008; Depoortere et al., 2008; Parola et al., 2006). Despite the abundance of Ae. mosquitoes in most of these areas, there was no evidence for significant autochthonous transmission of CHIKV. This may be in part due to the active surveillance of these imported cases, but may also indicate that the spread of the virus in the population was hampered by undetermined entomological or ecological reasons. Nevertheless, a recent study suggests that the vectorial competence of local Ae. albopictus is as efficient as the typical vector Ae. aegypti for CHIKV and DENV (Vega-Rua et al., 2013).

4.3.4.2. Autochthonous cases. Until 2007, CHIKV had never been known to circulate in Europe (MMWR 2006; 55: 1040–1042). In 2007, an outbreak of autochthonous chikungunya fever cases occurred in Italy. This outbreak was initiated by an individual returning to Europe from a visit to India (Angelini et al., 2007). The virus responsible for this outbreak was disseminated in the Emilia Romagna region of northern Italy by Ae. albopictus mosquitoes. More recently, in September 2010, an autochthonous transmission of CHIKV was recorded in southeastern France with two confirmed cases (Gould et al., 2010; Grandadam et al., 2011).

In conclusion, the epidemiological impact of recent chikungunya fever outbreaks has proved to be considerable in regions where the epidemic has spread. High seroconversion rates have been observed (commonly around 35% and up to 75%) and an enormous social and economic burden has been inflicted on affected communities (de Andrade et al., 2010; Ensereink, 2007; Krishnamoorthy et al., 2009; Queriaux et al., 2008). Moreover, despite the limited proportion of cases, outbreaks can have a significant impact on the health services due to the high proportion of symptomatic cases.

4.3.5. Nosocomial transmission of CHIKV

Since chikungunya fever is associated with extremely high and early viremia, the risk of blood-borne transmission of the virus has a high likelihood and interruption of blood supply for transfusion has been reported in affected countries to minimise the risk of transmission via infected blood products (Brouard et al., 2008; Liuimbruno et al., 2008; Ng et al., 2009b).

Although direct person-to-person transmission has not been reported, nosocomial transmission most probably can occur following needlestick injury, i.e. when the skin is accidentally punctured by a used (contaminated) needle. However, in the context of an outbreak, the nosocomial nature of transmission is difficult to establish. Interestingly, the only published case of documented nosocomial infection was reported in France, in a ‘cold’ epidemiological context associated with an imported case from Reunion island. It highlighted the risk of transmission for health care workers (Parola et al., 2006). During the CHIKV outbreak in Reunion island, corneas taken from asymptomatic donors proved to be infected with CHIKV and transmission of the virus occurred via this ocular route (Couderc et al., 2012).

5. Phylogenetic analysis

The first focused phylogenetic analysis of CHIKV identified three phylogroups based on the partial E1 viral structural glycoprotein sequence. The CHIKV strains represented in these different geographic lineages apparently circulate in regions that display different ecological backgrounds. The three genotypes are West-African, Asian, and East-Central-South-African (ECSA) (Powers et al., 2000; Schuffenecker et al., 2006).

The geographical distribution of the different genotypes has been described elsewhere (de Lambarlierie et al., 2008; Volk et al., 2010) and is summarised in Fig. 6. It is generally believed that the geographical origin of the virus is Africa, and this is supported by several observations (Ng and Hapuarachchi, 2010):

1. The most genetically divergent group of viruses is the West-African genotype;
2. The ECSA and Asian genotypes are paraphyletic in phylogenetic reconstructions (Cherian et al., 2009; Powers et al., 2000; Volk et al., 2010);
3. A complex sylvatic cycle has been known and reported for a longer period of time in Africa than in other regions and a larger number of vertebrate hosts and invertebrate vectors has been identified in Africa, i.e., propitious conditions for creating the observed viral genetic diversity;
4. More ancestral divergence times are estimated for West-African and ECSA genotypes compared with the Asian genotype (Cherian et al., 2009; Volk et al., 2010).

On the basis of recent in-depth phylogenetic analyses of available CHIKV isolates (Fig. 7), the evolutionary timescale of CHIKV was estimated to be during the past 300 years (Cherian et al., 2009; Volk et al., 2010). This supports a scenario in which sylvatic African CHIKV emerged as a human pathogen, probably during the 18th century, whereas all the currently identified epidemic genotypes (West-African, Asian, and ECSA) are estimated to have emerged during the 20th century.

The most important recent event is the spread of the ECSA lineages in the Indian Ocean and Asia. The strains form a monophyletic group within the ECSA lineages, with one branch including mostly Indian strains (and strains subsequently exported in various Asian, European and American countries) and the other strains identified in the South West Indian ocean islands (and strains subsequently exported). The progenitor of the viruses implicated was found to have existed 3 (Lo Presti et al., 2012; Volk et al., 2010) to 9 (Cherian et al., 2009) years before the 2004–2005 and subsequent epidemics arose.
During 2004–2005 chikungunya fever was transmitted to humans via *Ae. aegypti* (Arankalle et al., 2007) in Kenya, Comoros, Seychelles and some regions of Madagascar (de Lamballerie et al., 2008) but during the outbreak, a genetic change occurred at position 226 of the gene for the membrane fusion glycoprotein E1 with the substitution of a valine residue for an alanine (E1: A226V) (Schuffenecker et al., 2006). There is strong epidemiological and experimental evidence that this is an adaptive mutation that increases the viral replication in *Ae. albopictus* (Tsetsarkin et al., 2007). This mutation was reported to have occurred independently in different geographical locations and in different genetic backgrounds within the ECSA genotype (see the case of Gabonese strains in (de Lamballerie et al., 2008)). It represents an excellent example of convergent evolution (in the amino acid position 226) by different strains of CHIKV (de Lamballerie et al., 2008). However, it must be noted that the original, non-mutated *Ae. aegypti*-adapted strain was extremely efficiently transmitted in the regions where this mosquito was prevalent, with seroprevalences reaching more than 60% in Grande Comore and Lamu islands (see previous sections). Accordingly, it has been proposed that the emergence of the epidemic virus was linked with an initial adaptation to urban domestic *Ae. aegypti* mosquitoes (Ng and Hapuarachchi, 2010), a postulation further supported by compelling evidence of the urban transmission of the ECSA genotype of CHIKV in central Africa during the past decade (Pages et al., 2009).

The A226V adaptive mutation to *Ae. albopictus* increased the transmission by this mosquito (Tsetsarkin et al., 2009; Tsetsarkin et al., 2007) but marginally compromised the viral fitness in *Ae. aegypti* (Tsetsarkin et al., 2007; Vazeille et al., 2007). Despite its apparent high frequency, the A226V adaptive evolution did not occur in all CHIKV genetic backgrounds (it was limited to strains belonging to the ECSA genotype) and a complex pattern of other mutations may have been implicated in CHIKV adaptation to a specific *Ae.* spp. vector (Tsetsarkin et al., 2011a; Tsetsarkin et al., 2009). In particular, the E2-I211T substitution is suspected to have provided the background rendering possible the A226V mutation. It was reported to occur independently on three occasions (South Africa, 1976; Gabon, 2007; Kenya, 2004). However, whilst this mutation is present in the Asian genotype, the A226V mutation has never been observed in this genotype, and experimental evaporative states were employed to develop a more realistic model for the health burden associated with the increased incidence of chikungunya fever in the Eastern and Central African region.
mutagenesis failed to create efficient Ae. albopictus-adapted Asian genotype viruses that included this mutation.

Tsotsarkin et al. (2011a) demonstrated that lineage-specific epistatic interactions between substitutions at amino acid positions 226 and 98 of the E1 envelope glycoprotein have restricted the ability of endemic Asian CHIKV strains to adapt to Ae. albopictus. The same authors (Tsotsarkin and Weaver, 2011) identified an additional substitution, E2-L210Q which caused a significant increase in the ability of CHIKV to develop a disseminated infection in Ae. albopictus, but had no effect on CHIKV fitness in Ae. aegypti, or in vertebrate cell lines.

6. Pathogenesis of chikungunya fever

6.1. Target cells

Several recent publications have studied cell susceptibility to CHIKV replication. A panel of immortalised primary human cells was analysed and it was demonstrated that human epithelial and endothelial cells, primary fibroblasts and, to a lesser extent, monocyte-derived macrophages were susceptible to CHIKV infection whereas no replication was identified in lymphoid and monocytoïd cell lines, primary lymphocytes and monocytes, or monocyte-derived dendritic cells (Sourisseau et al., 2007a). CHIKV has also been isolated in a tremendous variety of continuous or diploid cell lines, including mammalian cells (Vero, BHK21, HEK-213T, MRCS, BGM, HeLa etc.), amphibian cells (XTC) and mosquito cells (C6/36, Ae, A20 etc.).

In animal models, CHIKV was detected in the cytoplasm of numerous mononuclear cells in the spleen and lymph nodes. Moreover, the sinusoidal endothelium of the liver and macrophages were identified as the main cellular reservoirs during the late stage of CHIKV infection in a macaque model (Labadie et al., 2010). In mouse models, fibroblasts constituted the main target cell of CHIKV in joint, muscle and dermal tissues, whilst in skeletal muscle cells CHIKV was rarely present in satellite cells. No infected leukocytes were detectable in the blood. In the central nervous system choroid plexus epithelial cells and ependymocytes were infected in contrast to microglial cells, astrocytes and microvascular endothelial cells. CHIKV was not found in placental tissue. In addition, the human syncytiotrophoblastic cell line BeWo was found to be refractory to infection (Couderc et al., 2008; Couderc and Lecuit, 2009).

In humans, viral growth in muscle satellite cells was detected whereas myoblasts were essentially refractory to infection (Ozden et al., 2007). In biopsies of skeletal muscles, joints and skin, CHIKV antigens were also detected but appeared to be confined to fibroblasts of the joint capsule of skeletal muscle fascia and of the dermis (Couderc et al., 2008; Couderc and Lecuit, 2009). CHIKV was also found (Hoarau et al., 2010a) in perivascular synovial macrophages in one chronic patient 18 months post infection (pi).

6.2. Animal models

6.2.1. Mice

Historically, the mouse was the model of choice to follow susceptibility to arbovirus infection, and was thus more often used for the study of the virus cycle than as a disease model. This is particularly clear for Sindbis virus (SINV), which induces arthritis in humans but was largely used as a model to study virus-induced encephalitis in mice. This highlights the main difficulty associated with the use of mouse models in assessing if they accurately mimic disease in humans both in terms of specificity and severity. A recent review was dedicated to this purpose (Teo et al., 2012).

Numerous studies were limited because the majority of adult wild-type (wt) mouse strains were asymptomatic following standard virus inoculation (intraperitoneal, intradermal, intravenous or subcutaneous (sc)). Thus, mainly neonatal mice or interferon (IFN)-α/β receptor knockout mice were used. These models and their derivatives (IFN-α pathway defective mice) (Rudd et al., 2012) have provided information about the innate immune response against alphaviruses and have been used for drug testing, since mortality rate and organ viral load can be used as convenient indicators of antiviral activity. (Couderc et al., 2009). The use of various mutants of IFN-α pathway defective mice enabled the mechanisms of the innate immune response specific to CHIKV to be deciphered (Couderc et al., 2008; Gardner et al., 2012; Schilte et al., 2012; Schilte et al., 2010; Wernke et al., 2011) implicating roles for Myd88, ISG15, IRF-3 and IRF-7 or viperine (Teng et al., 2012). Recently, a relative role of autophagy and the pathway in the host response against CHIKV was also assessed using Atg16L1/HM mice (Joubert et al., 2012).

More recently, A. Suhrbier and collaborators provided a model of chikungunya rheumatic disease in 6-week-old C57BL/6j mice by shifting the site of injection to the dorsal side of the footpad (Gardner et al., 2010). This subcutaneous (sc) injection route provided histological evidence of acute and persistence arthritis, tenosynovitis and myositis at the infected footpad, similar to that observed in RRV infected mice (Rulli et al., 2009, 2011). The acute phase was characterised by one-week viral replication peaking at 10^6 CCID50/ml and clear swelling and oedema of the inoculated foot (Gardner et al., 2010; Morrison et al., 2011). Tissue viral replication was detectable from day 1 to 21 in muscle, spleen lymph node and liver. In addition, in this adult mouse model, it was shown that mice initially infected with RRV are partially protected from superinfection with the CHIKV LR-2006 OPY-1 strain inoculated 4 weeks later. In mice, young age remained a factor of severity of the disease as is also observed in human babies but mechanisms of neurological disease are not perfectly compatible with these observations. Following intranasal infection of BALB/C or C57BL/6j mice, direct tissue necrosis is observed in the olfactory lobe but no direct infection of neurones was found (Teo et al., 2012). Finally in a recent study using IRF3/7(−/−) mice, it was demonstrated that inadequate IFN-α/β responses following virus infection can be sufficient to induce haemorrhagic fever and shock, a finding with possible implications for understanding severe CHIKV disease (Rudd et al., 2012).

Altogether, there is no current mouse model able to depict the long-lasting chronic arthralgic features found in up to 60% of the CHIKV-infected patient (Schilte et al., 2012 submitted) even if gene profiling of CHIKV arthritis in a mouse model reveals significant overlap with rheumatoid arthritis (Nakaya et al., 2012). However, mouse models are convenient and remain of specific interest for the in vivo screening of drugs targeting the acute phase of infection.

6.2.2. Nonhuman primates

NHP have been used extensively for studies of virus-induced pathology and evaluation of drugs and vaccines in a number of diseases (Vieboom et al., 2007; Walsh et al., 1996). Very early in the history of CHIKV studies it was found that NHP are susceptible to infection and are probably part of the natural reservoir in Africa and Asia (Inoue et al., 2003; Marchette et al., 1978; McCrae et al., 1971; Peiris et al., 1993). In field epidemiological studies in Central Africa (Central African Republic, Gabon) Cercopithecus and baboon monkeys have been used as sentinels to assess the presence of arboviruses in the wild (Saluzzo et al., 1980; Saluzzo et al., 1981; Saluzzo et al., 1982). African green monkeys and macaques were also used as sentinels for estimation of vector efficacy of different mosquito species (Jupp et al., 1981; Levitt et al., 1986; Turrell and Beaman, 1992; Turrell and Malinoski, 1992). These studies led to the identification of the major relevance of Ae. spp. in epidemic outbreaks.
The first experimental CHIKV infection was achieved in rhesus macaques (Macaca mulata) and bonnet macaques (M. radiata) in 1967, but only M. mulata showed episodes of fever within a few days of virus inoculation (Binn et al., 1967; Paul and Singh, 1968). The viral replication pattern in macaques was very similar to that reported in humans in 1965 with the same Asian strains, with plasma viremia found to be positive in macaques from days 1 to 6 and viral titres ranging from 3 to 5.3 log10 pfu/ml (Binn et al., 1967; Paul and Singh, 1968).

In these early experiments, little was recorded concerning the clinical signs associated with infection perhaps due to technical limitations. Amazingly, Paul and Singh in the 1968 study noted that: “the rate of infection in Aedes albopictus was consistently higher than that of Aedes aegypti when both species of mosquitoes were fed simultaneously on the same (viremic) macaque”. Despite this premonitory observation, Aedes albopictus was regarded as a minor potential vector of CHIKV, until the 2005–2006 Indian Ocean outbreak and its spread to India and South-East Asia. Subsequently, wild macaque susceptibility to infection was reported following epidemiological studies (Inoue et al., 2003; Peiris et al., 1993).

In the early 1990s, studies of CHIKV infection in NHP focused on macaque to mosquito transmission efficacy (Turell and Beaman, 1992; Turell and Malinoski, 1992). Recently, Roques and collaborators have infected cynomolgus macaques (Macaca fascicularis) using Reunion island strains of CHIKV and, by following biomarkers of CHIKV, found that replication was detectable during the first week of infection with a level of replication similar to human infection (Labadie et al., 2010). Performing in vivo titrations they demonstrated that as little as 10 pfu given IV, could produce infection in macaques, with viremic levels up to 10^6 pfu/ml being detected. At day 4 postinfection (pi) CHIKV is detected in the cerebrospinal fluid of all tested macaques but clinical neurological disease is detected only in macaques receiving the highest infectious doses. Interestingly, the acute infection seemed to be tightly controlled by poorly-characterised antiviral mechanisms given that the viral titre was reduced to basal levels at day 10 postinfection as described in humans or mice (Ziegler et al., 2008). These viral replication profiles were also recorded in rhesus macaques (Akahata et al., 2010; Chen et al., 2010).

In humans, early leukopenia was observed (Akahata et al., 2010; Borgherini et al., 2009; Borgherini et al., 2007; Labadie et al., 2010) together with markers of antiviral response (IFN-α/β) inflammation and cell immune activation (Higgs and Ziegler, 2010; Labadie et al., 2010). Whilst virus has been found in cerebrospinal fluid samples during the acute phase of infection, viral presence in the central nervous system is not clearly related to neuronal disease. It is more likely associated with non-specific inflammation that in rare cases might be associated with encephalopathy in animals infected with a very large amount of virus, as was probably the case for neonatal patients (Gerardin, 2010; Gerardin et al., 2008a; Labadie et al., 2010).

Recent studies in cynomolgus macaques demonstrated that CHIKV persists in target tissues after its clearance from the blood, as demonstrated by viral RNA detection using in situ hybridization assays (Labadie et al., 2010). At day 7 or 9, CHIKV is detectable in nearly every organ or compartment tested. Joints, secondary lymphoid organs and, to a lesser extent, muscles are affected and virus genetic material as well as isolation of replicative CHIKV is obtained up to 3 months pi. CHIKV replicates in several cell types during the acute phase (Higgs and Ziegler, 2010; Labadie et al., 2010) but is thereafter mainly detectable in macrophages, by immunohistochemical analysis (double staining). Infected monocytes and macrophages could be detected in the blood 6 h after infection (Roques et al., 2011) and in most tissues on the following day (in situ hybridization, immunohistochemistry, RT-PCR and virus isolation). Significant macrophage infiltration was also detected by histological analysis throughout the study and long after viral clearance from blood (Labadie et al., 2010).

This monocyte-macrophage tropism is consistent with the infection of human monocytes in vitro. These infected monocytes generate new viruses, which can be detected using HEK293 cells and titration assays, albeit at low levels (Her et al., 2010). Similarly, CHIKV can infect primary macrophages in vitro (Rinaldo et al., 1975; Sourisseau et al., 2007b) resulting in the production of highly variable amounts of virus, from 10^3 to 10^6 pfu/ml, regardless of the species being used for the studies (human, macaque, mouse) (Gardner et al., 2010; Labadie et al., 2010; Sourisseau et al., 2007b)(Fig. 8). These results are consistent with human studies, which reported that macrophages are susceptible to CHIKV infection both in vivo and in vitro (Hoarau et al., 2010b; Krejbich-Trotot et al., 2011). The alleviation of chikungunya-associated arthritis and myositis by treatment, with Bindarit, a MCP-1/CCL-2 inhibitor, in mice (Rulli et al., 2011) also strongly suggests that monocytes/macrophages, the main targets in MCP-1/CCL-2 tissue tropism, are central to muscle and joint disease (see below on the treatment section).

There is currently no animal model fully reproducing the chronic rheumatoid syndrome of chikungunya fever. Indeed, the disease picture in mice is mainly driven by destruction of tissues with huge cell infiltration (Gardner et al., 2010; Morrison et al., 2011; Rulli et al., 2011) and despite virus persistence, severe joint damage was not observed in macaques (Chen et al., 2010; Labadie et al., 2010).
et al., 2010). Nevertheless, both models suggest that inflammation, macrophage tissue tropism, and local viral persistence are involved in the establishment of chronic disease.

7. Vaccines

No vaccine against chikungunya is currently available. The first attempts to develop an inactivated vaccine were reported at the end of the 1960s and involved detergent treatment of virus (White et al., 1972). Unfortunately, these formalin-killed vaccines which showed promise appeared to be moderately immunogenic and limited to short time effects in a clinical trial (Harrison et al., 1971). As live attenuated vaccines are often more effective and, particularly for arboviruses, live vaccines are better at inducing rapid protection against homologous and heterologous challenge, the US Army developed a live, attenuated vaccine (TSI-GSD-218), based on an Asian 1970s strain, by serial passage in MRC-5 cells resulting in a virus (CHIKV 181/Clone 25), which carried significant genetic changes (Hoke et al., 2012; Levitt et al., 1986). This showed promise in protecting NHPs from challenge and was later tested in clinical trials showing good immunogenicity (Edelman et al., 2000; McClain et al., 1998). However, several adverse events were associated with TSI-GSD-218, the most severe being arthralgia in 8% of vaccinees.

Because of loss of information about the vaccine production, TSI-GSD-218 is no longer considered suitable for clinical use, but was used in proof of concept studies to evaluate the potential mechanisms of protection and resistance to the virus induced disease. Thus (Partidos et al., 2011) probed the attenuation and protective efficacy of this attenuated vaccine strain in mice with compromised IFN signalling. They showed that infection of A129 mice (defective in IFN-α/β and IFN-γ receptor signalling) with CHIKV 181/Clone 25 virus resulted in rapid mortality within 3–4 days. In contrast, all infected A129 mice (defective in IFN-α/β receptor signalling) survived with temporary morbidity characterised by ruffled appearance and body weight loss. A129 heterozygote mice that retain partial IFN-α/β receptor signalling activity remained healthy. Infection of A129 mice with CHIKV 181/Clone 25 virus induced significant levels of IFN-γ and IL-12 whilst the inflammatory cytokines, IFN-α and IL-6 remained low. A single administration of the CHIKV 181/Clone 25 vaccine strain provided both short-term and long-term protection (38 days and 247 days post-prime, respectively) against challenge with wt CHIKV–La Reunion (CHIKV–LR). This protection was at least partially mediated by antibodies since passively transferred immune serum protected both A129 and AG129 mice from wt CHIKV–LR and CHIKV 181/Clone 25 virus challenge.

These data were in accordance with others studies showing that IFN-α/β deficiency greatly exacerbates arthritic disease in mice infected with wt CHIKV (Couderc et al., 2008; Gardener et al., 2010), but not with the cell culture-adapted live-attenuated CHIKV 181/Clone 25 vaccine candidate (Gorchakov et al., 2012). The Weaver group showed that attenuation of CHIKV 181/Clone 25 vaccine strain is determined by two amino acid substitutions in the E2 envelope glycoprotein tested in two different murine strains: infant CD1 (5–6 days) and adult A129 mice (both defective in IFN pathway) (Gorchakov et al., 2012). Overall, these data highlight the importance of IFNs in controlling the CHIKV 181/Clone 25 vaccine strain, and demonstrate the ability of this vaccine to elicit neutralising antibody responses that confer short- and long-term protection against wt CHIKV–LR challenge.

7.1. Single recombinant antigens or Vero cell-adapted, formalin-inactivated vaccine

A vaccine based on recombinant envelope proteins of an Indian strain of CHIKV, adjuvanted with Alum, FCA or Montanide, elicited a balanced Th1/Th2 response with virus-neutralising antibodies in BALB/C mice, but the authors did not further challenge these mice (Khan et al., 2012). Kumar et al. (2012) evaluated recombinant E2 protein-based and whole-virus inactivated candidate vaccines, given intramuscularly, against CHIKV in BALB/c mice. They tested 4 adjuvants: Alum, Mw, CadB (rE2p), Alum/Mw (formalin-inactivated CHIKV) and Alum (BPL-inactivated CHIKV). Humoral immunity was assessed by ELISA and in vitro neutralisation test, using homologous and heterologous (Asian genotype) strains of CHIKV.

Two cohorts of vaccinated mice were challenged separately via the intranasal route with homologous virus 2 and 20 weeks after the second dose. Anti-CHIKV-antibody titres were dose dependent for all the immunogenic formulations. BPL-inactivated vaccines led to the highest ELISA/neutralising antibody (nAb) titres, whilst alum was the most effective adjuvant. In this adult BALB/c mouse/intranasal infection model (6–8 weeks), complete protection was observed following immunisation with the alum-adjuvanted rE2p, and both the inactivated vaccines, as no virus was detected in the tissues (muscle, brain, spleen) and blood (real time PCR) after challenge 2 weeks or 20 weeks-post-the second vaccine dose. However, with rE2p–CadB, very low viremia was recorded on the second day-post-challenge. Globally, a majority of vaccination trials in mice, even with the very simple inactivation system of whole CHIKV particles inactivated with aziridine (Brown, 2001), gave protective effect with as low as 0.01 µg of vaccine in C57BL6/ adult mice (12 weeks of age) with suppression of viremia and leg oedema.

7.2. Structural components of CHIKV in non-infectious virus-like particles (VLPs)

VLPs representing CHIKV envelope protein were produced in 293A renal epithelial cells and 293T human kidney cell lines infected by lentivirus vectors containing the subgenomic RNA sequence (C–E3–E2–6k–E1) (Akahata et al., 2010). The authors showed that, using VLP plus Ribi adjuvant, an antibody response 20 times higher than when using a DNA vaccine with the same sequence in Balb/C mice could be detected. Finally they reported protection in both mice (Ifnar −/−) and non-human primates challenged with the LR-2006 OPY-1 strain of CHIKV. However, the amount of produced VLPs remained very low in this system: from 0.1 to 20 mg/l within the 293T supernatant medium. The method was improved recently when the authors showed that a specific domain of the CHIKV E2 protein (amino-acid 233N–234K–252Q) regulated particle formation in human cells (Akahata and Nabel, 2012) and thus production of functional VLPs might be enhanced up to 200 fold.

A group from Wageningen University developed production of VLPs using chimeric baculovirus with the salmonid alphavirus (SAV) in insect cells and obtained functional processing and secretion (Metz et al., 2011). They recently developed such a process to obtain CHIKV VLPs within chimeric baculovirus. The first vaccination assays and virus challenge experiments were performed using the A Suhbrier mouse model system (Metz and Pijlman, 2011; Pijlman, 2012).

7.3. Vaccination with chimeric alphaviruses

Chimeric alphaviruses have been developed containing Venezuelan equine encephalitis virus (VEEV) or equine encephalitis virus (EEV) nonstructural protein coding sequences and CHIKV structural protein coding sequence with a programmed, attenuated, cell-type-restricted phenotype because of the lack of Old World alphavirus nsP2 or New World alphavirus capsid involved in innate cell response shutdown (Kim et al., 2011). To make these viruses incapable of transmission by mosquito vectors and to differentially...
regulate the expression of viral structural proteins, their replication was made dependent on the internal ribosome entry sites (IRES), derived from the encephalomyocarditis virus IRES (Plante et al., 2011).

The design of the genomes was complemented by selection procedures, which adapted viruses to replication in tissue culture and produced variants which (i) demonstrated different levels of replication and production of the individual structural proteins, (ii) efficiently induced the antiviral response in infected cells, (iii) could not replicate in mosquito cells (Darwin et al., 2011), and (iv) efficiently replicated in Vero cells. Finally, this vaccine candidate was tested in highly sensitive A129 mice (IFN-γ/β R−/−) and was found not deleterious in contrast to CHIK 181/Clone 25 vaccine strain but efficiently induced a protective immune response (Wang et al., 2011b) and was able to provide cross-protection for alphaviruses of the Semliki Forest antigenic complex (Partidos et al., 2012).

Using the same procedure and CHIKV vaccine candidate, Partidos et al. induced a cross-protective immunity in adult AG129 mice (IFN-α/β R−/−; IFNγ R−/−) against OVVN 9 weeks post vaccine (Partidos et al., 2012). The authors also showed that transplacental passive anti-CHIKV antibody transfer to 3 week old A129 offspring from the vaccinated dam was protective against a OVVN intra-dermal challenge.

Using a completely different strategy, GenPhar developed a recombinant adenovirus CadVax-CHIKV containing codon optimised sequence containing the CHIKV structural genes (C–E3–E2–6k–E1). This vaccine was tested using the A Subhier mouse model (Wang et al., 2011a). At 5 weeks post-vaccination, the reciprocal of the neutralisation titre (RNT) was close to 5 × 10⁵ which was similar to the titre observed after infection with the wild type CHIKV (100% protection against cytopathic effect of 200 CICD₅₀ of virus). The mice were challenged 45 days after vaccination and were shown to be fully protected against both foot swelling and viral replication (undetectable compared to 5 × 10⁶ CICD₅₀ in control group).

Other recombinant viruses tested in mice include chimeric vesiculoalphavirus, in which the entire CHIKV envelope polyprotein was expressed (E3–E2–6k–E1) (Chattopadhyay et al., 2013). These VSV-G-CHIKV chimeras were attenuated in tissue culture, propagated to high titre without VSV-G complementation and shown to protect adult mice C57BL/6 challenged 34 days postvaccination by the sc route of inoculation in the left rear foot-pad (Morrison model). Efficiency of vaccination was assessed for neutralising antibodies using the plaque reduction neutralisation test (PRNT) 10 × 10⁶ PBMCs, but they did not challenge the animals. Thus, all candidate vaccines except one (TSA-GSD-218) have so far only been tested in mice, and the studies have mainly focused on neutralising antibodies and not on cellular responses. From the literature, it is unclear whether any of these vaccines have proceeded into human clinical trials.

In addition, recent studies have shown that (1) the recognised protective immune response against CHIKV consists predominantly of IgG3 antibodies and (2) the early neutralising IgG response to CHIKV in infected patients targets a dominant linear epitope on the E2 glycoprotein (Kam et al., 2012a; Kam et al., 2012b). However, another study reported that CHIKV is able to escape the impact of neutralising antibodies by direct cell-to-cell transmission (Lee et al., 2011). This observation implies that an effective vaccine cannot be assessed purely by its ability to induce detectable neutralising antibodies.

In conclusion, whilst it is evident that an effective vaccine against CHIKV infection is clearly needed, inactivated vaccines are unlikely to provide strong and long-lasting immunity, and they will almost inevitably require multiple doses, thus reducing their cost effectiveness. DNA vaccines, thus far, have proved less effective in humans than in other mammals. Live, attenuated alphaviruses are being used as the genetic backbone for vaccines under development by Chiron, Alphavax and others and this approach is the most likely to lead to an effective vaccine with long-lasting immunity for CHIKV. However, it remains to be determined whether the inevitable high development costs of a safe and effective live, attenuated chimaeric vaccine against CHIKV will prove to be a restraining influence.

7.4. Electroporated DNA

A plasmid containing codon and RNA-structure-optimised E3–E2–E1 protein sequences without the 6k peptide but including the furin cleavage sites as well as a Kozak-IgE leader sequence at the 5’ end of the genome, was generated by GeneArt (Regensburg, Germany) and vaccination was performed by 3 subsequent electroporations into quadriceps from 8-week-old female BALB/c mice (Mallilankaraman et al., 2011). One week after the third electroporation CHIKV neutralising antibodies were significantly enhanced with a RNT median of 320 (which produced 100% protection against cytopathic effect using a challenge dose of 100 CICD₅₀ of virus) and a high CHIKV specific cellular response was demonstrated as measured by IFN-γ ELISPOT assay against E1 and E2 peptide pools (1613 ± 170 SFC/10⁶ cells). This construction thus induced an improved immune response, when compared with the previous C–E1–E2 construction from the same group (Muthuman et al., 2008). At this time mice were challenged by intranasal exposure with 107 pfu (25 µl) of the CHIKV-PC-08 strain (ECSA genotype).

In summary, the mice were protected from the high morbidity normally associated with intranasal infection (i.e., spongiform lesion apoptotic bodies, microglial nodules in the external granular layer of the cerebral cortex; severe myocardial and liver degeneration/necrosis) which required euthanasia of control mice at day 6–12 pi. Despite marked weight loss during the first 3 days, vaccinated mice returned to normal values thereafter. However, in the vaccinated animals sacrificed at day 5 pi, some microglial oedema was seen in the brains suggesting that immunisation decreased CHIKV-mediated pathology but did not fully protect the brain in the intranasal inoculation model. In contrast, the disease in mice vaccinated with DNA expressing only the CHIKV capsid induced a morbidity picture identical to the control non-immunised mice. Finally Mallilankaraman et al., 2011 tested the immune response induced following this vaccination strategy in four rhesus macaques (5 immunisations). Two weeks later, they detected variable neutralising antibody responses (RNT 80–1200) and specific T cell responses (200–500 SFC/10⁶ PBMCs), but they did not challenge the animals. Thus, all candidate vaccines except one (TSA-GSD-218) have so far only been tested in mice, and the studies have mainly focused on neutralising antibodies and not on cellular responses. From the literature, it is unclear whether any of these vaccines have proceeded into human clinical trials.

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In conclusion, whilst it is evident that an effective vaccine against CHIKV infection is clearly needed, inactivated vaccines are unlikely to provide strong and long-lasting immunity, and they will almost inevitably require multiple doses, thus reducing their cost effectiveness. DNA vaccines, thus far, have proved less effective in humans than in other mammals. Live, attenuated alphaviruses are being used as the genetic backbone for vaccines under development by Chiron, Alphavax and others and this approach is the most likely to lead to an effective vaccine with long-lasting immunity for CHIKV. However, it remains to be determined whether the inevitable high development costs of a safe and effective live, attenuated chimaeric vaccine against CHIKV will prove to be a restraining influence.

7.5. Attenuation of CHIKV by large-scale codon re-encoding

This novel method of attenuating viruses (Burns et al., 2006; Coleman et al., 2008; Mueller et al., 2010; Mueller et al., 2006; Song et al., 2012) was recently applied to the LR2006 CHIKV strain (ECSA genotype); the nucleic acid composition of large coding regions was modified, without modifying the encoded proteins, by introducing a large number of synonymous mutations (Nougairede et al., 2013). Using reverse genetic methods, synonymous mutations were randomly introduced in three regions of around 1.4 kb that encode structural (nsP1 and nsP4) and non-structural
(E1–E2) proteins. Up to 882 synonymous mutations were introduced representing, 7.5% of the complete genome of the virus.

The replicative fitness of the resulting re-encoded viruses was studied in cellulo using primate and mosquito cells. Introducing a large number of presumably slightly deleterious synonymous mutations significantly decreased the replicative fitness of CHIKV in both primate and arthropod cells and this diminution correlated directly with the degree of re-encoding giving the possibility to modulate precisely the degree of fitness loss. The use of several re-encoded regions located throughout the viral genome reduces the likelihood of complete phenotypic reversion due to recombination between wild-type and re-encoded CHIKVs. In addition, the stability of these re-encoded viruses was assessed by serial passaging of these viruses 50 times either in primate or insect cells or in each cell line alternately. The analysis of these passaged viruses showed that it exhibited a stable phenotype, and that the response to codon re-encoding was largely compensatory in nature, with little reversion of mutations.

Studies in animal models are obviously needed to evaluate the potential of these re-encoded CHIKVs for producing vaccine candidates. The important message is that generating an attenuated strain of CHIKV by large-scale re-encoding represents a potentially important route to vaccine development, because this method does not modify amino acid sequences and therefore potentially alleviates the likelihood of novel phenotypic properties, and also provides the advantages of all the live attenuated vaccines, including reduced costs and single dose induction of long-term immunity.

8. Antiviral therapy

Several drugs are known to be effective against CHIKV when tested in vitro, but no recognised antiviral treatment is currently available.

8.1. Experimental therapies targeting steps in viral replication

8.1.1. Antibody-based therapies

Passive immunotherapy has been used for over a century in the treatment of viral infectious disease. In a recent study, monkeys were immunised with a vaccine based on CHIKV VLPs. The immunoglobulin G (IgG) extracted from the sera of these monkeys was administered passively to mice, which were then infected with CHIKV. The immune IgG protected the mice from infection by CHIKV (Akahata et al., 2010). Human immunoglobulins purified from plasma of CHIKV convalescent patients also exhibited a high neutralising activity and a powerful prophylactic and therapeutically efficacious CHIKV infection in mice (Couderc et al., 2009).

More recently, human recombinant monoclonal antibodies exhibited strong and specific neutralising activity in vitro when tested against a wide variety of CHIKV strains and were able to significantly delay the CHIKV-driven lethality in AGR129 mice (IFN-α/β/γ R−/− and RAG-2−/−) (Fric et al., 2013; Warter et al., 2011). Passive immunotherapy may constitute an efficacious prevention strategy and treatment for individuals exposed to CHIKV who are at risk of severe infection, such as neonates born to viremic mothers and adults with chronic underlying medical conditions (Couderc et al., 2009).

8.1.2. Interferon

The IFN system is capable of inhibiting virus infections in the absence of adaptive immunity. Treatment of cells with IFN-α/β upregulates the expression of several hundred genes which, in combination, specify the antiviral state. No single gene is pivotal and, for any given virus, a subset of genes is probably required to limit viral replication. Several of the upregulated genes encode enzymes that have been studied intensively, such as dsRNA-dependent protein kinase R (PKR), 2′,5′-oligoadenylate synthetase (OAS) and MxA (Randall and Goodbourn, 2008). The most important current application of IFN therapy as an antiviral treatment is for the chronic phase of viral infections, such as HCV and HBV (Heim, 2012). Nevertheless, IFN therapy has also been proposed for acute viral infection such as SARS or influenza (Wang and Fish, 2012).

IFN is known to inhibit the growth of other viruses within the genus Alphavirus (Łukaszewski and Brooks, 2000; Pinto et al., 1990). In the case of SINV, it was found that the balance between type I IFN induction and the ability of the virus to develop further rounds of infection is determined in the first few hours of virus replication, when only low numbers of cells and infectious virus are involved (Frolov et al., 2012). In another study, an emerging alphavirus in salmonid aquaculture called Salmonid alphavirus-3 (SAV-3), was sensitive to the preinfection antiviral state induced by IFN-α, whilst IFN treatment with IFN-α was not able to limit viral replication (Xu et al., 2010). This is consistent with recent studies in mice, in which it was found that IFN-α treatment prevented arthritis due to CHIKV only if given before infection. Since such a situation would not exist in most cases of human infection, IFN-α is not likely to prove useful for therapy (Gardner et al., 2010).

8.1.3. Small-molecule drugs

8.1.3.1. Ribavirin. Ribavirin, the nucleoside analogue 1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, exhibits antiviral activity against a variety of RNA viruses in cell culture through at least three distinct mechanisms: inhibition of the cellular protein isomerase monophosphate dehydrogenase (IMPDH), immunomodulatory effects, and incorporation as a mutagenic nucleoside by the viral RNA polymerase. All three activities may play an antiviral role in vivo (Crotty et al., 2002).

Ribavirin is known to inhibit in cellulo a large range of RNA viruses and notably viruses in the genus Alphavirus, such as Semliki forest virus (SFV) and CHIKV (Ho et al., 2010; Smee et al., 1988). In animal models, ribavirin is effective against a more limited set of viruses, mainly RNA viruses. In humans, ribavirin is currently used in combination with interferon-α to treat hepatitis C virus (HCV) infections and it has been used as monotherapy for Lassa fever and severe respiratory syncytial virus (RSV) infections (Crotty et al., 2002).

Some in vivo antiviral activity of ribavirin during chikungunya fever has been reported recently in a non-randomised human cohort, but no statistical analysis was available due to the small number of participants (Ravichandran and Manian, 2008). The combination of interferon and ribavirin has been found to exert a synergistic antiviral effect against two alphaviruses (CHIKV and SFV) in vitro (Briolant et al., 2004).

8.1.3.2. Chloroquine. Chloroquine and its hydroxy-analogue, hydroxychloroquine are weak bases that are known to affect acid vesicles leading to dysfunction of several enzymes. Some viruses enter their target cells by endocytosis in the lysosomal compartment, where the low pH, along with the action of enzymes, disrupts the viral particle, thus liberating the infectious nucleic acid and, in several cases, enzymes necessary for viral replication. For some enveloped viruses, post-translational modification of the envelope glycoproteins also occurs within the endoplasmic and trans-Golgi network vesicles, and involves proteases and glycosyl-transferases, some of which require a low pH. Thus it was shown that chloroquine/ hydroxychloroquine can impair the replication of several viruses by interacting with the endosome-mediated viral entry or the late stages of replication of enveloped viruses (Savarino et al., 2003).
The potential for in cellulo application of chloroquine as an antiviral treatment was first reported more than 40 years ago (Inglot, 1969), but in the case of alphaviruses, and notably SFV, it was also reported that chloroquine enhanced virus replication in mice (Maheshwari et al., 1991). In vitro inhibition of CHIKV replication by chloroquine has been reported (Delogu and de Lamballerie, 2011) and should be distinguished from the anti-inflammatory effect previously reported in patients at the chronic stage of infection (Brighton, 1984). In 2006, a double-blind, placebo-controlled randomised trial evaluating the efficacy of chloroquine against chikungunya fever did not identify a significant difference between the chloroquine and placebo groups, in terms of either the mean duration of febrile arthralgia or the decrease of viremia from day 1 to 3. Moreover, at day 200 postinfection, patients treated with chloroquine declared more frequently that they still suffered from arthralgia than patients who had received a placebo (De Lamballerie et al., 2008).

In conclusion, whilst in vitro inhibition of CHIKV replication is strongly established, the narrow therapeutic index and the absence of obvious biological or clinical improvement during a clinical trial do not argue in favour of a curative use for chloroquine in noncomplicated cases of chikungunya fever (Delogu and de Lamballerie, 2011). This suggests that previous similar favourable results obtained in cellulo only with chloroquine for highly pathogenic viruses, such as the SARS-CoV (Keyaerts et al., 2004), should be considered carefully.

8.1.3.3. Arbidol. The antiviral drug Arbidol (ARB) (1-methyl-2-phenyl-thiomethyl-3-carboxoxy-4-dimetylaminomethyl-5-hydroxy-6-bromoindolehydrochloride monohydrate) was developed by the Centre for Drug Chemistry, Moscow for use against respiratory viruses, such as the SARS-CoV (Keyaerts et al., 2004), should be considered carefully.

8.1.3.4. Furin inhibitors. Alphavirus envelope glycoproteins are initially produced first as precursors (E3E2 or p62) and during virion maturation further cleaved at short multibasic motifs. Amongst cellular proteases, the basic amino acid-specific furin or furin-like proprotein convertases (PCs) (Voss et al., 2010) have been shown to be involved in such a calcium-dependent process, resulting in the cleavage of surface glycoproteins (Ozden et al., 2008). Since it was demonstrated that uncleaved glycoprotein precursors induce defects in viral production for other alphaviruses such as SINV and SFV, a recent study tested the hypothesis that an irreversible furin-inhibiting peptide (decanoyl-RVKR-chloromethyl ketone (dec-RVKR-cmk)) could inhibit CHIKV infection (Ozden et al., 2008). Like other alphaviruses, the envelope glycoproteins of CHIKV are initially produced as precursors that are further cleaved by furin-like proprotein convertases (PCs) (Voss et al., 2010). dec-RVKR-cmk induced in cellulo stronger inhibition of viral infection than chloroquine when added immediately following infection. A combination of both drugs induced an additive effect, which was further enhanced when the drugs were added to the cells before the virus (Ozden et al., 2008).

8.1.3.5. Structure-based-specific inhibitors. Crystal structures of the replicative proteins of CHIKV and several other alphaviruses have been reported (Lampio et al., 2000; Malet et al., 2009; Russo et al., 2006; Voss et al., 2010) and provide new opportunities to develop structure-based, specific inhibitors. Using these principles, Singh et al. (2012) identified four lead compounds in a large library of compounds that potentially could inhibit the nsP2 protease of CHIKV, based on a homology model of the crystal structure of the nsP2 protein of VEEV. More recently, Lucas-Hourani et al. (2012) identified, amongst 3040 molecules, one natural compound (ID1452-2) that inhibits CHIKV replication in vitro by interfering with nsP2 activity.

8.1.3.6. Other inhibitors of virus replication. A series of complex polycyclic molecules isolated from a rare native plant of New Caledonia (Trigonostemon cherrieri), Trigocherrins 1,2 and 6 and Trigocherrinolides 7–9, have recently been tested in cellular assays against CHIKV, SINV and SFV. Comparisons of selectivity indices led to the conclusion that currently Trigocherrins are the most potent identified inhibitors of CHIKV replication. The mechanism of inhibition has not yet been determined (Allard et al., 2012a; Allard et al., 2012b). More recently, another species of the same genus (Trigonostemon howii) collected in central Vietnam has also proved to be a potent inhibitor of CHIKV (Bourjot et al., 2012a).

Similarly, products extracted from the leaves of the Madagascar plant Anacolosa pervilleana, demonstrated moderate activity in vitro against CHIKV replication (Bourjot et al., 2012b; Bourjot et al., 2012c). More recently, Kaur et al. (2013) identified amongst a highly purified natural product compound library, a cephalotaxine alkaid called harringtonine that displayed potent inhibition of CHIKV infection affecting CHIKV RNA production as well as viral protein expression with minimal cytotoxicity.

8.1.4. Antisense oligonucleotides and siRNA

Peptide-conjugated phosphorodiimdate morpholino oligomers (PPMO) are nucleosie resistant and water-soluble single-stranded-DNA-analogues that can enter cells readily and act as steric-blocking antisense agents through stable duplex formation with complementary RNA (Stein, 2008). When applied in cellulo, PPMO decreased viral tirtles by several orders of magnitude, and reduced viral replication and/or increased survival of mice experimentally infected with poliovirus, coxsackievirus B3, dengue virus, West Nile virus, respiratory syncytial virus, Ebola virus, influenza A virus and the alphavirus VEEV (Paessler et al., 2008; Stein, 2008).

Small interfering RNA (siRNA) is an ancient, highly conserved and natural mechanism that is induced by double-stranded RNA. It defends cellular genomes against infection by viruses and transposons. siRNA specifically degrades RNA in the cytoplasm of eukaryotic cells (Hannon, 2002). It has been successfully utilised to inhibit replication of many RNA and DNA viruses (influenza, human immunodeficiency virus, Japanese encephalitis virus, West Nile virus, foot and mouth disease virus, hepatitis B virus, and herpes simplex virus) and notably alphaviruses such as VEEV and SFV (Dash et al., 2008).

In 2008, Dash et al. (2008) designed two siRNAs that bind to conserved regions of the CHIKV genome (nsP3 and E1). When added to cells up to 72 h postinfection, these siRNAs produced significant viral inhibition, reducing both the infectious virus titre and the total viral load. In another study, siRNA was used to inhibit the autophagy machinery in CHIKV-infected cells (siRNA against the transcript of Beclin 1, an autophagic protein). This procedure substantially decreased CHIKV replication (Krejci-Trotot et al., 2011). More recently, plasmid-based small hairpin RNA (shRNA)
against CHIKV E1 genes has provided significant inhibition of infectious virus production in vitro. Strong and sustained anti-CHIKV protection was also conferred in suckling mice pretreated with shRNA E1 (Lam et al., 2012).

8.2. Experimental therapies targeting host responses to infection

8.2.1. Sphingosine kinase 1 activity

In a recent review, Carr et al. summarised the information available regarding sphingosine kinase 1 (SphK1) activity against virus infections (Carr et al., 2012). SphK1 has been proposed as a potential therapeutic agent for the treatment of cancer, immunological and inflammatory disorders. SphK1 catalyses the phosphorylated form of sphingosine to generate sphingosine-1-phosphate (S1P). The SphK1/S1P axis has well described roles in cell signalling, viz., the cell death/survival decision, the production of a pro-inflammatory response, immunomodulation and control of vascular integrity. The authors argued that the clinical picture of chikungunya fever is similar to the painful joint syndrome of rheumatoid arthritis (RA). Accordingly, SphK1/S1P could play a significant role in infections by RNA viruses. Indeed, data suggest that raising S1P systematically, or reducing SphK1 activity and S1P locally in joints, does appear to have beneficial effects in clinical cases of RA. Moreover S1P lyase inhibitors are under investigation for RA

8.2.2. Inhibitor of monocyte chemotactic protein (MCP)

The mechanisms by which arthropigenic alphaviruses such as CHIKV cause disease are poorly understood, but the inflammatory response to infection contributes to viral elimination from the blood and clinical recovery at both the acute stage and the chronic form of the disease (Dupuis-Maguiraga et al., 2012). It has also been suggested that macrophages play a critical role in the inflammatory response (Dupuis-Maguiraga et al., 2012).

An inhibitor of monocyte chemotactic protein (MCP) synthesis, called Bindarit, was recently shown to be a potent anti-inflammatory drug, which does not cause systemic immunosuppression or affect arachidonic acid metabolism (Rulli et al., 2009). Clinical trials have confirmed the positive safety of Bindarit and its oral availability (Rulli et al., 2009). In two recent studies, Bindarit treatment improved the articular symptoms of CHIKV and RRV disease in mice and reduced tissue destruction, without affecting the viral load in the tissues (Rulli et al., 2009; Rulli et al., 2011).

In summary, no antiviral drug is currently available for the treatment of standard presentations of chikungunya fever. Chloroquine, which has been used in the past, should not be utilised at the acute phase of the disease, based on data collected during a prospective clinical trial in Reunion Island. In severe presentations, ribavirin may be proposed, but very limited information is available and the precise indications of the treatment and the therapeutic protocol (e.g., doses and duration) have not been established. However, on a more positive note, current investigations may lead to the identification of new antiviral candidates, as an increasing number of molecules are being associated with a clearly defined mechanism of viral inhibition in cell-based systems, and show significant activity in animal models. Therapeutic protocols for severe cases of chikungunya fever may also be established, based on specific immunoglobulins or molecules that can interfere with some aspects of the inflammatory response associated with CHIKV infection.

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