Biology and pathogenesis of chikungunya virus

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Abstract | Chikungunya virus (CHIKV) is a re-emerging mosquito-borne alphavirus responsible for a recent, unexpectedly severe epidemic in countries of the Indian Ocean region. Although many alphaviruses have been well studied, little was known about the biology and pathogenesis of CHIKV at the time of the 2005 outbreak. Over the past 5 years there has been a multidisciplinary effort aimed at deciphering the clinical, physiopathological, immunological and virological features of CHIKV infection. This Review highlights some of the most recent advances in our understanding of the biology of CHIKV and its interactions with the host.

Chikungunya fever, an arboviral disease that is caused by chikungunya virus (CHIKV) and is transmitted by mosquitoes, was first recognized in epidemic form in East Africa in 1952–1953 (REFS 1,2). 'Chikungunya' is a Makonde word meaning 'that which bends up' and refers to the contorted posture of infected patients suffering from severe joint pain[3]. During the past 50 years, numerous CHIKV re-emergences have been documented in both Africa and Asia, with irregular intervals of 2–20 years between outbreaks[4]. The absence of serological surveillance means that precise numbers of individuals infected during these outbreaks can only be estimated. In 2004, CHIKV emerged in Kenya and spread to Comoros, where 5,000 cases were reported[5]. In 2005–2006, the outbreak spread to other islands in the Indian Ocean, including La Réunion; this was the first time that CHIKV had infected an occidental country. La Réunion, which is part of France, is an island in the Indian Ocean with a population of ~785,000; strikingly, an estimated 300,000 cases of CHIKV infections[5,6] and 237 resultant deaths[7] were reported. Viral genetic analysis supported the link between the infections in La Réunion and the outbreak in Kenya in 2004 (REFS 8,9). The epidemic also spread to India, where it is estimated that more than 1.5 million people were infected, and it was subsequently identified in Europe and the United States, where it is thought to have been imported by infected travellers returning from areas with high incidence rates. Indeed, between July and September 2007 the virus caused the first autochthonous epidemic outbreak in the north-east of Italy, with more than 200 human infections all traced back to the same index case[10–14]. Currently, chikungunya fever has been identified in nearly 40 countries (FIG. 1), and in

2008 it was listed as a US National Institute of Allergy and Infectious Diseases (NIAID) category C priority pathogen[4,15]. Recent epidemic re-emergences were also documented in Kinshasa, the Congo (50,000 estimated cases in 1999–2000)[16], Indonesia (2001–2003)[17], the Indian Ocean islands of Mayotte, Seychelles, Mauritius and La Réunion[6] (300,000 cases in 2005–2006), India (1.4–6.5 million estimated cases in 2006–2007)[10,18,19], and Malaysia and Thailand (3,000 and 42,000 estimated cases in 2009, respectively, according to the CDC), to name a few (FIG. 1).

CHIKV is a member of the family *Togaviridae*, genus *Alphavirus*[20], which comprises enveloped, positive-stranded-RNA viruses. In humans, CHIKV infection is of rapid onset and is typically cleared in 5–7 days. For reasons that are still being explored, the ongoing outbreak has been marked by severe symptoms[21]. The case fatality rate has been estimated to be 1 in 1,000, with most deaths occurring in neonates, adults with underlying conditions and the elderly[22–27]. Notably, these are the first documented deaths attributed to CHIKV infection.

In response to the public health need, and because La Réunion is a French territory, researchers in the Institut Pasteur, Paris, France, teamed up with clinicians in La Réunion and other countries to create a CHIKV task force. This multidisciplinary team has been working together for the past 5 years to analyse the epidemiology, physiopathology, virology, entomology and host response to infection. In this Review we highlight the recent major advances achieved not only by the CHIKV task force, but also by numerous other teams in the arbovirus community that have worked to address this important re-emergent infectious disease (see BOX 1).

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Box 1 | **A multidisciplinary approach to addressing public health problems**

The chikungunya virus (CHIKV) outbreak in La Réunion highlighted the importance of using a multidisciplinary approach to address medical and public health issues. Numerous teams in the arbovirus community have rapidly focussed their studies on CHIKV. One noticeable initiative was the creation of a CHIKV task force comprising virologists, epidemiologists, entomologists, pathologists, immunologists and clinicians working in La Réunion. Epidemiologists and virologists defined new mutations that emerged during the 2005–2006 outbreak. Clinicians from several medical specialties studied the clinical features of infection in neonates. Entomologists and virologists characterized the vector change from *Aedes egyptii* to *Aedes albopictus*. All of the groups worked together to define viral tropism in humans and animal models. This progress, together with a collaboration with key industrial partners, resulted in the development of new tools for the diagnosis of CHIKV infection and the availability of new information regarding the treatment and management of individuals susceptible to severe disease.

Such multidisciplinary efforts are uncommon in life sciences. A major barrier to the multidisciplinary approach is the specialization required to train highly focused professionals, who in turn often construct a 'boundary' around their area of expertise. This has resulted, in many cases, in the development of jargon that cannot be understood by other scientists, even those in closely related fields. Although this 'specialist' approach has led many technological and conceptual advances over the past 50 years, it could be argued that the complex nature of disease pathogenesis requires a team-based approach to problem solving. In addition, the advent of '-omics' research has resulted in the generation of a plethora of data that cannot be integrated and applied by an individual research unit. Physicists, chemists and even clinicians have all understood the need for collaborative, multidisciplinary approaches. The rapid response to the CHIKV outbreak on the part of the scientific community shows that the power of collaborative science can extend to public health and life sciences. This was only a first step, as additional work will be required to identify new treatments and prophylactic strategies against this pathogen.

CHIKV pathogenesis

The genus Alphavirus contains approximately 30 members, which probably diverged a few thousand years ago[28,29]. Some alphaviruses are not pathogenic to humans, whereas others are highly infectious, with the associated clinical diseases ranging from mild to severe. Alphaviruses can be broadly divided into New World and Old World viruses[30,31]. These two groups have evolved distinct ways of interacting with their respective hosts and differ in their pathogenicity, tissue and cellular tropism, cytotoxicity and interference with virus-induced immune responses. It should be noted that most alphaviral infections in humans and domesticated animals are considered a 'dead end' — that is, the virus cannot be transmitted to a new host, so the evolutionary pressures driving viral diversification may be linked to their true host species. For CHIKV, a thorough exploration of other zoonotic viral reservoirs has not been carried out.

From a clinical perspective, the two groups of alphaviruses are subdivided into those associated with encephalitis (predominantly New World viruses) and those associated with polyarthritis and a rash (predominantly Old World viruses)[29,32,33]. Although CHIKV is a member of the arthritogenic alphaviruses, during the recent outbreak there were documented cases of meningoencephalitis (primarily in neonates) and haemorrhagic disease[22], indicating that these signs are important sequellae of acute CHIKV infection[32,34,35]. Unlike typical encephalogenic alphaviruses, which infect neurons, CHIKV seems to infect the stromal cells of the central nervous system and, in particular, the lining of the choroid plexus (FIG. 2).

Transmission of CHIKV occurs through a bite by infected *Aedes aegypti* or *Aedes albopictus*, although in the recent epidemic some cases were the result of maternal–fetal transmission[22]. Following transmission, CHIKV replicates in the skin and then disseminates to the liver and joints, presumably through the blood[36–38] (FIG. 2). The incubation period is 2–4 days and is followed by a sudden onset of clinical disease with no prodromal phase (FIG. 3). Symptoms of CHIKV infection include high fever, rigors, headache, photophobia and a petechial rash or maculopapular rash. In addition, most infected individuals complain of severe joint pain that is often incapacitating[39–41] (see also the WHO guidelines on the clinical management of chikungunya). 'Silent' infections (infections without illness) do occur but are
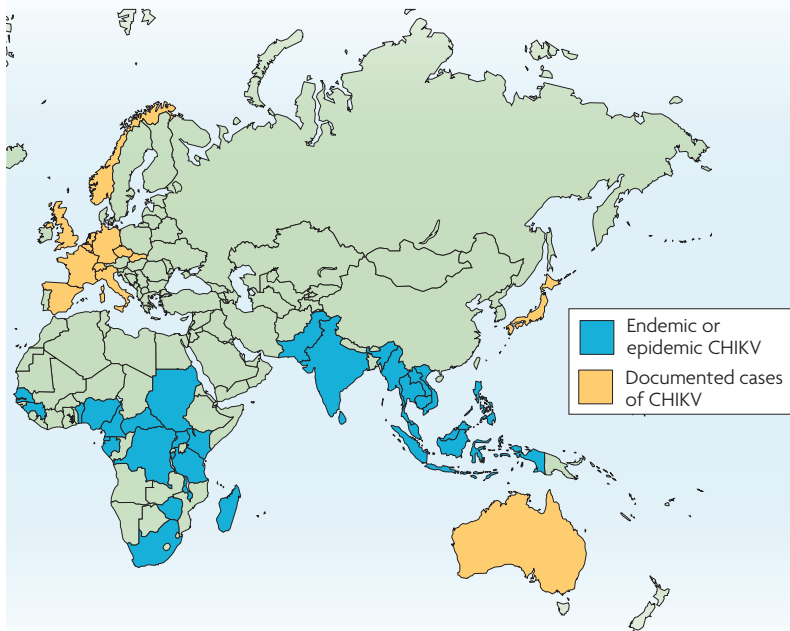

Figure 1 | **Worldwide distribution of chikungunya virus.** Both blue and yellow indicate countries where cases of chikungunya fever have been documented, and blue indicates countries where chikungunya virus (CHIKV) has been endemic or epidemic. Figure is modified, with permission, from REF. 4 © (2007) Society for General Microbiology.

Legend:
- Endemic or epidemic CHIKV
- Documented cases of CHIKV

rare, being observed in around 15% of infected individuals[21]. Strikingly, during the acute phase, the viral load can reach 10^8 viral particles per ml of blood, and the plasma concentration of type I interferons (IFNs) is in the range of 0.5–2 ng per ml, accompanied by a robust induction of other pro-inflammatory cytokines and chemokines[42–44] (FIG. 3).

The acute phase of CHIKV infection typically lasts from a few days to a couple of weeks. In contrast to the acute phase, the chronic phase of disease has not been extensively investigated. Recurrent joint pain, which can last for years in some cases, is experienced by 30–40% of those infected, although this is not thought to be a result of chronic infection, as infectious virus cannot be isolated from these patients. Radiographic studies are typically normal or show mild swelling, which is consistent with joint pain. It has been suggested that this joint pain, similarly to the pain caused by the related alphavirus Ross River virus (RRV)[45], is immune mediated. This has not been formally shown, although the presence of autoantibodies has been reported in one case of CHIKV infection with severe musculoskeletal complications[46].

Figure 2 | **Dissemination of chikungunya virus in vertebrates.** Transmission of chikungunya virus (CHIKV) occurs following a mosquito (*Aedes aegypti* or *Aedes albopictus*) bite. CHIKV then replicates in the skin, in fibroblasts, and disseminates to the liver, muscle, joints, lymphoid tissue (lymph nodes and spleen) and brain. The target cells are indicated for each tissue.

Cellular and tissue tropism

A large effort has been made recently to describe viral tropism and replication in cell culture systems and in animal models to better understand CHIKV pathogenesis (for details on the Alphavirus life cycle in mammalian cells, see BOX 2). Studies in the 1960–1980s showed that CHIKV grows in a panel of non-human cell lines, including Vero cells, chick embryo cells, BHK21 and L929 fibroblast-like cells, and HEp-2 hepatic cells[47–50]. The cellular tropism of CHIKV in humans was characterized recently. In tissue culture experiments, the virus replicates in various human adherent cells, such as epithelial and endothelial primary cells and cell lines, fibroblasts and, to a lesser extent, monocyte-derived macrophages[51]. CHIKV also replicates in human muscle satellite cells, but not in differentiated myotubes[52] (FIG. 2). In contrast to adherent cells, B cells and T cells are not susceptible to CHIKV infection *in vitro*[51,53]. Like other alphaviruses, CHIKV is highly cytopathic in human cell cultures, and infected cells rapidly undergo apoptotic cell death[33,51]. This pattern of replication probably governs the pathological properties of the virus.

In a highly pathogenic mouse model in which animals lack the type I IFN receptor (*Ifnar*⁻/⁻ mice) and are much more susceptible to severe disease, the CHIKV tissue tropism seems to match the tropism reported using *in vitro* systems. CHIKV was found to primarily target muscle, joint and skin fibroblasts, but it was also identified in the epithelial and endothelial layers of many organs, including the liver, spleen and brain[38] (FIG. 2). Notably, newborn and young mice are highly sensitive to CHIKV infection and represent a valuable model for studying CHIKV pathogenesis[38,54].

Non-human primates have also been used as models for CHIKV-associated pathology and vaccine testing[55–57]. In two recent studies, intravenous or intradermal CHIKV inoculation of macaques resulted in high viraemia, peaking 24–48 hours after infection. Although infection was not lethal, it was associated with a transient acute lymphopenia and neutropenia (that is, loss of lymphocytes and neutrophils, respectively), an increase in monocytes and a pro-inflammatory response[56,57]. Infection recapitulated the viral, clinical and pathological features observed in humans[57]. CHIKV targeted lymphoid tissue, the liver, the central nervous system, joints and muscle during the acute phase[57]. Persistent infection (measured 44 days post-infection) occurred in splenic macrophages and in endothelial cells lining the liver sinusoids. Tissue derived from these animals carried low levels of replication-competent virus[57]. It will be important to establish whether this is reflective of the situation during human infection and what role viral persistence has in the chronic sequellae associated with chikungunya fever. One recent study has indicated that elderly patients are at high risk of chronic disease, but clearly more work is necessary[58].

The human tissue culture systems and the simian and mouse models have provided clues about the tissue and cellular localization of CHIKV in infected humans. Samples from CHIKV-infected patients with myositic syndrome showed CHIKV antigen expression in skeletal
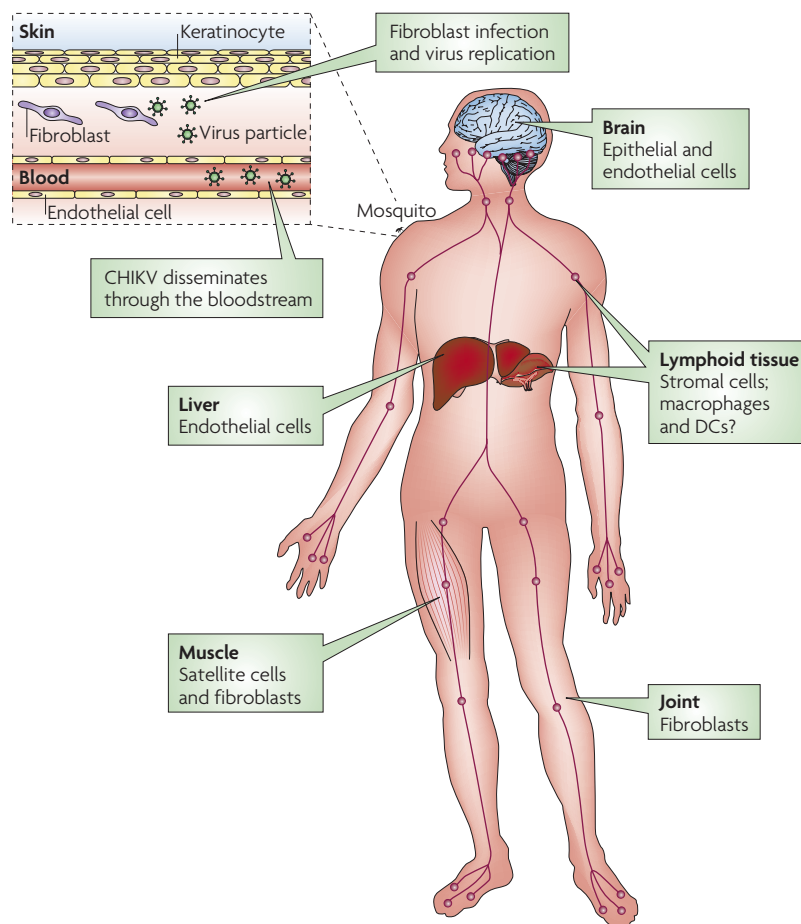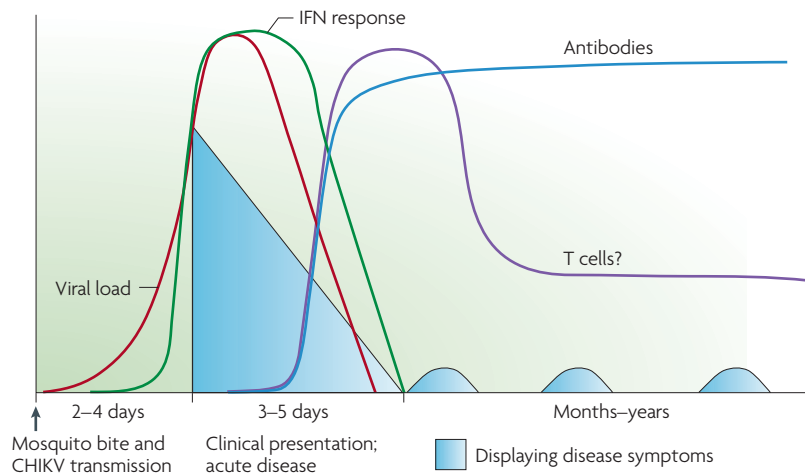
Figure 3 | Chikungunya virus pathogenesis. Following transmission by mosquito bite, infected individuals experience an acute onset of disease 2–4 days after infection. Symptoms include high fever, rigors, headache and a petechial or maculopapular rash. In addition, most infected individuals complain of severe joint pain that is often incapacitating. Disease onset coincides with rising viral titre, which triggers the activation of an innate immune response, the hallmark of which is the production of type I interferons (IFNs). Patients successfully clear the virus approximately 1 week after infection, and only at this time is there evidence of CHIKV-specific adaptive immunity (that is, T cell and antibody-mediated responses). Importantly, ~30% of individuals experience long-term sequellae that include arthralgia and, in some cases, arthritis.

muscle satellite cells but not in muscle fibres[52]. Infected fibroblasts have also been reported in biopsy material taken from acutely infected patients[38]. There is a debate about the sensitivity of primary blood monocytes to CHIKV infection[51,59]. Sourisseau et al.[51] reported that the high viral load in blood plasma (ranging from 10^5 to 10^8 RNA copies per ml) during acute infection does not correspond to detectable levels of viral RNA in blood cells. They also found that, in vitro, peripheral blood mononuclear cells (including B cells, T cells and monocytes) are not susceptible to CHIKV infection[51]. By contrast, Her et al.[59] observed that CHIKV antigens are detected in vitro in monocytes exposed to high viral inocula (multiplicity of infection = 10–50). CHIKV antigen-positive monocytes were also isolated from acutely infected patients[59], but definitive evidence of productive infection was not established. As monocytes are phagocytic, and as viral titres are high in acutely infected patients, the presence of negative-strand viral RNA must be assessed to determine whether productive infection of monocytes does occur and whether monocytes are true targets of CHIKV. There are notable cell tropism variations among alphaviruses, which probably influences the pathogenesis of disease[30]. For example, human monocyte-derived dendritic cells (DCs) and plasmacytoid DCs (pDCs) are not sensitive to CHIKV[51,60]; Venezuelan equine encephalitis virus (VEEV) can infect DCs and macrophages in lymphoid tissues and cultures, whereas this is not the case for Eastern equine encephalitis virus (EEEV)[61,62]. Interestingly, EEEV infection of myeloid-lineage cells is restricted after virus binding and entry, by inhibiting translation of incoming EEEV genomes[61]. Of note, RRV

infects mouse macrophages[31,63–65], which are implicated in the pathogenesis of disease. During RRV infection, infiltrates of inflammatory macrophages are observed in muscles and joints[45], and treatment of mice with agents that are toxic to macrophages abrogated the symptoms of infection[66].

The cellular tropism of alphaviruses is regulated by many parameters. For example, RRV envelope glycoproteins allow the infection of mouse DCs but not human DCs[67], and the ability of Sindbis virus (SINV)[68] and VEEV[69] to infect DCs is determined by a single amino acid substitution in the E2 envelope protein. Further work should examine the sensitivity of Langerhans cells to CHIKV and other alphaviruses. The use of rhabdoviruses and lentiviruses pseudotyped with CHIKV envelope glycoproteins may facilitate the study of early entry or post-entry events[70].

Type I IFNs (IFNα and IFNβ) are also major regulators of tissue tropism and virulence[71]. For example, they prevent the widespread dissemination of Semliki Forrest virus (SFV) in mouse extraneural tissues, and this is associated with reduced sensitivity to type I IFNs and enhanced virus pathogenicity[72]. More generally, type I IFN induction in vivo, as well sensitivity to type I IFN treatment in cell culture, differs markedly between different alphaviruses[73]. The interplay between CHIKV and the innate immune system is discussed below.

Jumping species — an atypical vector for CHIKV

CHIKV is endemic to Africa, India and Southeast Asia and is transmitted to humans by several species of mosquito, with geographical variations[33,74–76]. Although A. aegypti is the classical vector for CHIKV, the 2005 outbreak in La Réunion was associated with an atypical vector, A. albopictus[6,14,75–78]. Other Aedes species are sensitive to experimental CHIKV infection, but their role in field transmission has not been shown[79].

Why did CHIKV adopt A. albopictus as its host? The transmission success of arboviral diseases depends on many factors, including the geographical and temporal distribution of the insect vectors, their growth rate and the viral incubation period inside them[80–84]. A. albopictus is a competent vector for dengue virus and numerous arboviruses, and its distribution has expanded recently, even replacing A. aegypti in some places[14,83–85]. It is native to Southeast Asia and has colonized both tropical and temperate regions. It was identified in Europe (first in Albania) and in North America in the early 1980s, probably having been introduced through shipments of used car tyres from Asia[86]. Currently, A. albopictus is present in at least 12 European countries and in around 25% of the United States.

There are several features of A. albopictus that make it a good viral vector: it survives in both rural and urban environments; it was probably first zoophilic and then progressively became anthropophilic[87]; it is long lived (4–8 weeks); it has a flight radius of 400–600 metres; and it can successfully infect humans and animals because it is aggressive, quiet and diurnal. Furthermore, the mosquito's eggs are highly resistant and can remain viable throughout the dry season, giving rise to larvae and adults the

Box 2 | The alphavirus life cycle
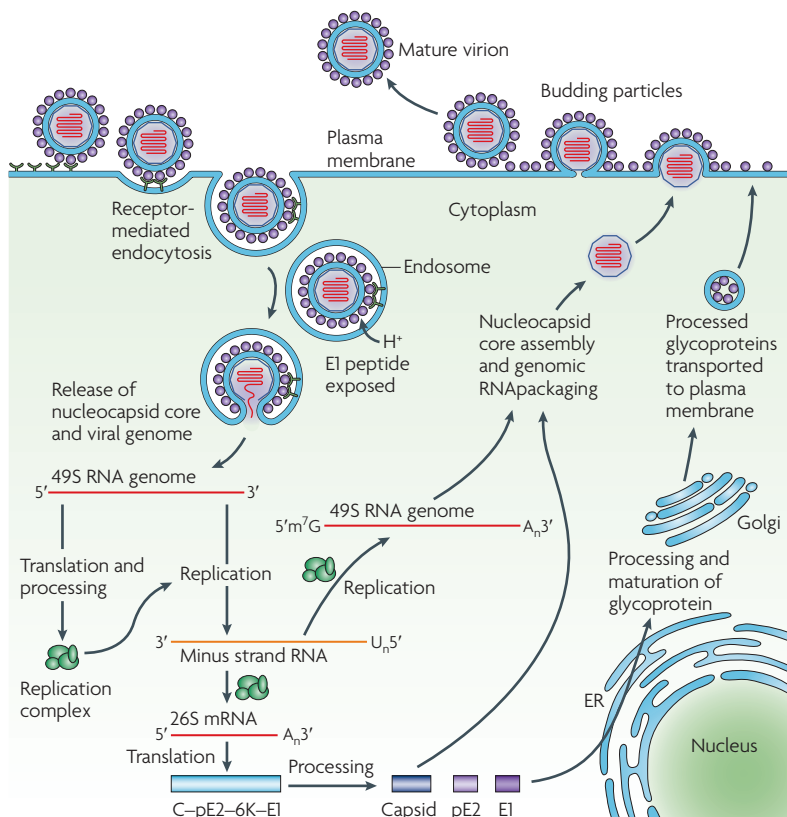
The Alphavirus life cycle is depicted in the figure. Alphaviruses enter target cells by endocytosis[33]. A few receptors (for example, dendritic cell-specific ICAM3-grabbing non-integrin 1 (DC-SIGN; also known as CD209), liver and lymph node-SIGN (L-SIGN; also known as CLEC4M), heparan sulphate, laminin and integrins) have been implicated in this process, but their precise roles have not been firmly established[33]. Following endocytosis, the acidic environment of the endosome triggers conformational changes in the viral envelope that expose the E1 peptide[90,135], which mediates virus–host cell membrane fusion. This allows cytoplasmic delivery of the core and release of the viral genome[6,29,136]. Two precursors of non-structural proteins (nsPs) are translated from the viral mRNA, and cleavage of these precursors generates nsP1–nsP4. nsP1 is involved in the synthesis of the negative strand of viral RNA and has RNA capping properties[33,137], nsP2 displays RNA helicase, RNA triphosphatase and proteinase activities and is involved in the shut-off of host cell transcription[138], nsP3 is part of the replicase unit and nsP4 is the viral RNA polymerase[33]. These proteins assemble to form the viral replication complex, which synthesizes a full-length negative-strand RNA intermediate. This serves as the template for the synthesis of both subgenomic (26S) and genomic (49S) RNAs. The subgenomic RNA drives the expression of the C–pE2–6K–E1 polyprotein precursor, which is processed by an autoproteolytic serine protease. The capsid (C) is released, and the pE2 and E1 glycoproteins are generated by further processing. pE2 and E1 associate in the Golgi and are exported to the plasma membrane, where pE2 is cleaved into E2 (which is involved in receptor binding) and E3 (which mediates proper folding of pE2 and its subsequent association with E1). Viral assembly is promoted by binding of the viral nucleocapsid to the viral RNA and the recruitment of the membrane-associated envelope glycoproteins. The assembled alphavirus particle, with an icosahedral core, buds at the cell membrane.

following rainy season. All of these features of *A. albopictus* provided CHIKV with a great opportunity to infect humans once it had adopted this mosquito species as its host. In fact, the human–mosquito–human transmission cycle was so efficient that there was no identified animal reservoir during the epidemic in La Réunion[76].

How was CHIKV able to efficiently adapt to *A. albopictus*? An extensive genomic analysis of recent clinical CHIKV isolates from the Indian Ocean outbreak identified unique molecular features when compared with the few previously available sequences from laboratory-adapted CHIKV[6]. In particular, changes were observed in E1 — a class II viral fusion protein that mediates viral entry at low pH[88–90] — potentially affecting viral fusion, assembly and/or cell tropism. Notably, a specific mutation in E1 (Ala226Val) was absent in the initial viral strains but was observed in >90% of the later strains[6]. Interestingly, in the related alphavirus SFV the amino acid residue at position 226 regulates cholesterol dependency during the virus–host cell fusion process[91]. The efficiency of alphaviral entry depends on host cell membrane composition (including the levels of cholesterols, which mosquitoes obtain through blood meals). A mutation that affects cholesterol dependency could improve the ability of CHIKV to infect insect cells by providing a better adaptation to the lipid composition of these cells. Indeed, experimental infection of *A. albopictus* showed that the early viral strains were not as successful at replicating in this mosquito as later, mutated viruses[75,76]. The E1 Ala226Val mutation is directly responsible for a substantial increase in CHIKV infectivity for *A. albopictus* and leads to more efficient viral dissemination into mosquito secondary organs and transmission to suckling mice[75]. Both early and late viruses invaded salivary glands in a similar pattern, but the crossing of the midgut epithelium, one of the primary sites of infection[75,76,92], was a crucial step that made *A. albopictus* particularly susceptible to later CHIKV isolates[76]. Interestingly, this mutation has no effect on viral replication in *A. aegypti*[75]. Moreover, the E1 Ala226Val mutation facilitates viral replication in cholesterol-depleted C6/36 mosquito cells[75]. Other mutations that have been identified recently in E2 also regulate CHIKV adaptation to its mosquito hosts[93]. Whether the enhanced ability of later CHIKV isolates to invade *A. albopictus* relates to cholesterol dependency has not been proved yet, but these observations strongly suggest that the rapid evolution of CHIKV conferred a selective advantage on the virus to infect and replicate in *A. albopictus*. Of note, both early and late CHIKV isolates replicated similarly in various human cells[51] and in the non-human BHK21 cell line[75].

In summary, the adaptive mutation of the virus to replicate in *A. albopictus*, which is more common than *A. aegypti* in some geographical regions and can act as an efficient vector for CHIKV, facilitated the spread of CHIKV. This, together with the fact that the human population had not previously encountered CHIKV and was therefore immunologically naive[84], contributed to the magnitude of the La Réunion CHIKV epidemic.

Immune control of CHIKV

Epidemiological data from the CHIKV outbreak in La Réunion indicate that >85% of individuals harbouring antibodies for CHIKV reported symptoms of infection[21]. Although precise information regarding CHIKV transmission is difficult to obtain, the

epidemiological data indicating that one-third of the island's inhabitants became infected suggest that CHIKV is highly successful. Humans, however, are not defenceless, and in fact CHIKV is efficiently cleared within 4–7 days of infection[94–96] (FIG. 3). As a typical adaptive immune response (for example, CHIKV-specific B cell and T cell activation) requires at least 1 week to develop, the innate immune system seems to be capable of controlling CHIKV. Below we discuss the innate and adaptive immune responses that are known to control CHIKV infection.

Innate immune control of CHIKV. From an immunological perspective, CHIKV and type I IFNs share a common history. Isaacs and Linemann[97] first described IFN as a substance with antiviral activity in 1957. CHIKV was discovered only 5 years earlier owing to a major chikungunya fever epidemic that lasted from the late 1950s to 1964 in Asia and South India[98]. It was at this time that the study of CHIKV intersected with the study of type I IFNs — in 1963, Gifford and Heller[99] reported in *Nature* that chick embryo fibroblasts infected with CHIKV produced detectable levels of type I IFNs 3 hours after infection. Despite a series of high-profile publications in 1963–1970 (including REFS 100,101), the study of CHIKV was subsequently eclipsed by that of other model microorganisms.

Work over the past 50 years has defined type I IFNs as central to the control of viral infection. IFNα and IFNβ are mainly produced by leukocytes and fibroblasts, respectively. The production of type I IFNs is triggered by pattern recognition receptors (PRRs), which detect conserved molecular motifs — termed pathogen-associated molecular patterns (PAMPS) — including surface glycoproteins, single-stranded (ss) or double-stranded (ds) RNA and unmethylated CpG-containing DNA[102,103]. Two types of PRRs that recognize viral PAMPS have been identified: Toll-like receptors (TLRs; which reside in the plasma membrane or the endosomal compartments) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs; which reside in the cytoplasm)[104,105]. The TLRs comprise 11 transmembrane proteins, 6 of which (TLR2, TLR3, TLR4, TLR7, TLR8 and TLR9) are known to be involved in antiviral immunity[106]. TLR2 and TLR4 can be activated by viral surface glycoproteins (for example, haemagglutinin of measles virus)[107–110]; TLR7 and TLR8 are triggered by ssRNA (for example, that of influenza virus)[111]; TLR3 is engaged by extracellular dsRNA[112]; and TLR9 is activated by unmethylated CpG-containing DNA (for example, that of herpes simplex virus)[110]. RLRs include RNA helicases (such as MDA5 (melanoma differentiation-associated protein 5; also known as IFIH1), RIG-I and PKRs (dsRNA-dependent protein kinases); these detect viral RNA in the cytoplasm[113]. As CHIKV is a ssRNA virus that replicates with a dsRNA intermediate, potential sensors include TLR3, TLR7, TLR8 and the RLRs (FIG. 4).

The mechanisms underlying type I IFN production following CHIKV infection were recently characterized. Previous data had shown that CHIKV does not directly infect primary leukocytes[51], but it was expected that a ssRNA virus would be able to directly activate haematopoietic cells, especially pDCs. This assumption is based on the fact that pDCs express TLR7 and the observation that they can respond to viral PAMPs even in the absence of infection[114]. Remarkably, *in vitro* CHIKV infection of human peripheral blood mononuclear cells as well as of human and some mouse DC subsets indicate that this virus does not directly engage PRRs for the induction of type I IFNs[60]. Instead, using *in vitro* and *in vivo* studies, it was shown that type I IFNs are produced by infected fibroblasts[60]. The production of type I IFNs by infected fibroblasts is regulated by CARDIF (CARD adaptor inducing IFNβ; also known as MAVS), which acts downstream of MDA5 and RIG-I, and may involve ssRNA detection by both RLRs (FIG. 4). On the basis of the CHIKV tissue tropism (FIG. 2), it has been argued that CARDIF is engaged in infected fibroblasts and stromal cells. However, adult
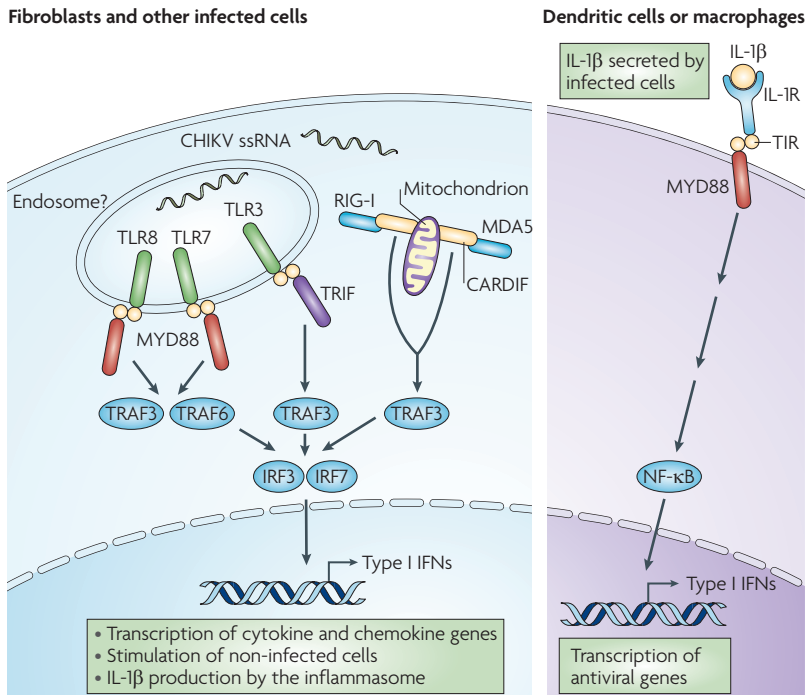

Fibroblasts and other infected cells

Dendritic cells or macrophages

Figure 4 | **Innate immune control of chikungunya virus.** Chikungunya virus (CHIKV) is a single-stranded RNA (ssRNA) virus and may generate double-stranded RNA intermediates during replication that have the potential to engage the pathogen recognition receptors Toll-like receptor 3 (TLR3), TLR7 and TLR8 and the retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) melanoma differentiation-associated protein 5 (MDA5) and RIG-I. These receptors activate a signalling cascade that leads to the activation of type I interferons (IFNs) and the transcription of cytokines and chemokines. Recent evidence suggests that the production of type I IFNs by infected fibroblasts and other cell types is regulated by the adaptor protein CARDIF (CARD adaptor inducing IFNβ; also known as MAVS), which acts downstream of MDA5 and RIG-I. The inflammasome may also induce IL-1β production by infected cells (not shown). In a mouse model, protection was also partly dependant on the TLR adaptor myeloid differentiation primary response protein 88 (MYD88). This may suggest a role for TLRs, possibly on haematopoietic cells. In addition, MYD88 also acts as an adaptor for interleukin-1β receptor (IL-1R), which could be activated by the secretion of IL-1β from infected cells, thereby inducing type I IFN in non-infected cells. IRF, IFN regulatory factor; NF-κB, nuclear factor-κB; TIR, Toll/IL-1 receptor domain; TRAF, tumour necrosis factor receptor-associated factor; TRIF, TIR domain-containing adaptor protein inducing IFNβ.

Cardif^{-/-} mice infected with CHIKV had only a subtle phenotype, suggesting that other sensors must also be involved in the host response to CHIKV. Indeed, in addition to induction by the RLR pathway, protection may also be mediated by myeloid differentiation primary response protein 88 (MYD88), which is an adaptor protein for several TLRs and for the interleukin-1β (IL-1β) receptor (FIG. 4). As *Cardif*^{-/-} and *Myd88*^{-/-} mice were not as susceptible to CHIKV infection as *Ifnar*^{-/-} mice, RLR and TLR recognition of CHIKV may cooperate for rapid clearance of the infection.

Two possible pathways may account for the role of MYD88 in the control of CHIKV infection. As stated above, haematopoietic cells are poorly stimulated by CHIKV, suggesting that the virus does not engage TLRs in a conventional manner[59,60]. There is, however, the possibility that endosomal TLRs are engaged as a result of haematopoietic cells phagocytosing infected cells, the latter being a source of viral PAMPs. For example, infection by SFV results in the generation of dsRNA that may engage TLR3 on CD8+ DCs following engulfment[115]. A second possible means of engaging MYD88 relates to its role as an adaptor for the IL-1β and IL-18 receptors[116]. There has been a surge of new information regarding the role of the inflammasome, which is well recognized as being crucial for IL-1β production following bacterial infection and also seems to participate in the control of viruses[117,118]. As such, IL-1β produced by CHIKV-infected cells following inflammasome activation may participate in viral control by stimulating non-infected cells in a MYD88-dependent manner[43,60] (FIG. 4).

The activation of PRRs triggers the production of type I IFNs, which are crucial for antiviral immunity. Indeed, mice lacking IFNAR are much more susceptible to severe chikungunya fever than wild-type mice[38]. Interestingly, using bone marrow chimaeras of wild-type and *Ifnar*^{-/-} mice, it has been shown that type I IFNs mainly target non-haematopoietic cells, such as stromal cells, to achieve viral clearance[60].

Type I IFNs, in turn, activate the transcription of interferon-stimulated genes (*ISG*s), as evidenced in infected humans, who have high levels of *ISG* products in the plasma[42]. *ISG*s contain promoter elements that are sensitive to interferon response factors (IRFs)[119]. There are >300 ISG proteins encoded in our genome and, although the function of most is unclear, those that are well characterized have been shown to have crucial roles in host defence[120]. The antiviral roles of ISG proteins have been defined for several related viruses. The most extensively studied is SINV, which can be controlled by RNase L[121], ISG15 (REF. 122), ISG49, ISG54, ISG56 (REF. 123), ZAP (also known as ZC3HAV1)[124] and serpins[125]. For CHIKV, only one ISG involved in viral control has been defined so far: it has been reported that HeLa cells transfected with 2′, 5′-oligoadenylyl synthetase 3 (OAS3) are more resistant to CHIKV replication[126]. It remains unclear how OAS3 blocks CHIKV replication, but initial studies suggest that its function does not depend on its downstream effector, RNAse L[126]. Other ISG proteins are probably also involved in innate immune responses to CHIKV.

Similarly to other viruses, CHIKV is likely to have evolved mechanisms to modulate both the induction of type I IFNs and the effector molecules stimulated by type I IFN signalling pathways. On the basis of data from other Old World alphaviruses such as SINV and SFV, one candidate for this immune modulation is non-structural protein 2 (nsP2), which acts as an inhibitor of host protein synthesis[127,128]. Future studies will be required to decipher the crosstalk between the different ISGs involved in the innate immune response to CHIV and to determine the ISGs that are necessary for (as opposed to just capable of) inhibiting CHIKV replication.

Adaptive immune responses following CHIKV infection. Given the acute nature of CHIKV infection and disease pathogenesis, and the urgent need to tackle the spreading epidemic, there has so far been little effort afforded to understanding the sequellae of chronic infection with CHIKV and the role of the adaptive immune system in protection from subsequent re-infection. In fact, a deeper understanding of the humoral (that is, antibody-mediated) and cell-mediated immune response is important, as it is relevant for vaccine development and may impinge on our understanding of the chronic joint pain experienced by 30–40% of CHIKV-infected individuals.

One study showed that serum from donors in the convalescent phase contains neutralizing CHIKV-specific immunoglobulins[129]. Strikingly, it is possible to protect *Ifnar*^{-/-} mice by administering these immunoglobulins, suggesting that sterilizing immunity is an achievable goal. Consistent with this interpretation, when CHIKV infection preceded the administration of CHIKV-specific immunoglobulins by 24 hours, the mice were no longer protected from lethal infection. Such passive immunity has been shown for other alphaviruses and may indeed be a viable medical intervention, especially in those individuals susceptible to severe CHIKV infection, such as neonates.

Even less is known about the role of lymphocytes during disease pathogenesis. One marked effect of CHIKV infection is acute lymphopenia. It has been reported that 80% of 157 individuals with acute CHIKV infection experienced a decrease in the frequency of circulating B cells and T cells. Nearly half of those individuals had lymphocyte levels that were one-quarter of the lower limit for healthy individuals[130]. This was probably not a direct effect of the virus on lymphocytes, as CHIKV does not infect B cells and T cells. Instead, it is possible that type I IFNs induce cell death in lymphocytes, as they do in other acute infections. In addition, upregulation of stromal IFN-stimulated chemokines (for example, CXC-chemokine ligand 10 and CC-chemokine ligand 5) can trigger the migration of lymphocytes from the blood to the tissues, leading to lymphopenia[131]. In most CHIKV-infected individuals, repopulation of the circulating pool of lymphocytes occurs soon after resolution of infection. Interestingly, RAG-deficient mice

Inflammasome
A molecular complex of several proteins that, following assembly, cleaves pro-interleukin-1 (pro-IL-1), thereby producing active IL-1.

Sterilizing immunity
An immune response that leads to the complete removal of the pathogen.

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(which lack lymphocytes) can clear CHIKV infection (C. Schilte & M.A., unpublished observations), suggesting that lymphocytes are not crucial for immunity during acute infection. However, this observation must be interpreted with caution, as mice are not the natural hosts of CHIKV. Nonetheless, the kinetics of viral clearance and the absence of data regarding exacerbated disease in humans with weakened adaptive immunity (for example, individuals infected with HIV) suggest that the innate arm of the immune response is sufficient for clearance of the infection in humans as well.

The role of cytotoxic T lymphocytes (CTLs) in particular during alphavirus infection has barely been studied so far. A dominant mouse CTL epitope present in a conserved region of the capsid of Old World alphaviruses has been described[64], strongly suggesting that CTLs can be induced by CHIKV. Whether CTLs participate in the elimination of CHIKV-infected cells in humans remains to be addressed.

One side effect of adaptive immune responses is the possible induction of autoimmunity, caused by cross-reactivity between viral and host antigens. Again, there is little information on this subject, but there is certainly a possibility that B cell and T cell responses to CHIKV are implicated in the long-term joint disease experienced by many convalescent patients[46]. More information and careful epitope mapping are needed to determine whether some of the clinical findings of CHIKV infection are caused by autoimmune reactivity.

Vaccine development. The initiative to stimulate protective immunity as a strategy for preventing CHIKV infection in humans began in the early 1970s. Two formulations showed early promise: formalin fixation and ether extraction were both successful means of inactivating CHIKV while maintaining its ability to stimulate the production of haemagglutination-inhibiting, complement-fixing and neutralizing antibodies[44,132]. These initial studies included human trials, with 16 army recruits receiving formalin-fixed CHIKV vaccine prepared in bank-frozen green monkey kidney tissue culture[132]. Work progressed slowly, but the US Army remained committed to this effort and in 2000 carried out a Phase II clinical trial examining the safety and immunogenicity of the use of live attenuated CHIKV vaccine[55,133,134]. A 1962 strain of CHIKV from an outbreak in Thailand was used in this case, and the vaccine was formulated as a lyophilized supernatant from human MRC-5 cells. Of the 58 study subjects that received the vaccine, all developed neutralizing antibodies, and 5 subjects experienced mild to moderate joint pain[134].

One important issue that arose during these early studies is the potential interference arising from sequential administration of vaccines specific for heterologous alphaviruses. Specifically, individuals vaccinated against VEEV showed poor neutralizing-antibody responses to the CHIKV vaccine[133]. Similarly, vaccination with CHIKV followed by VEEV resulted in reduced VEEV-specific responses[133]. This is a concern,

as the populations at risk for these agents live in overlapping geographical regions.

Following the recent epidemic, there has been a renewed effort for vaccine development. A new formulation using virus-like particles has been shown to induce neutralizing antibodies in macaques[56]. These antibodies offered protection following challenge with different strains of CHIKV, and transfer of the macaque antisera into highly susceptible *Ifnar*$^{-/-}$ mice protected the mice from infection[56]. This approach may prove useful, not only for vaccination against CHIKV, but also for vaccination against other pathogenic alphaviruses.

Conclusion

An unprecedented effort teaming up clinicians, virologists, immunologists, molecular biologists and entomologists throughout the world has considerably furthered out understanding of CHIKV biology. Viral replication has been extensively studied in mammalian and insect cell culture systems. Biological samples from acutely and chronically infected humans have been analysed and, together with the development of animal models, have provided invaluable tools for studying the physiopathology of infection. CHIKV shares many characteristics with other Old World alphaviruses but also displays unique and previously unexpected properties.

Important questions remain to be addressed. The relative roles of the virus and the immune system in acute and chronic pathologies associated with CHIKV infection have yet to be deciphered. Analysing the impact of the adaptive immune response on controlling infection will have implications for the development of vaccine strategies. At the cellular and molecular levels, identifying additional members of the array of sensors involved in viral detection will bring new insight into the interaction of the virus with the innate immune system. From a virological standpoint, the role of non-structural viral proteins as well as the identity of cellular receptors allowing viral entry are partly unknown.

Perhaps one sad reality that we must reflect on is that CHIKV research received so much support as a direct result of the epidemic having emerged in an occidental country — an island that is part of France. Weekly articles in the lay press documented the escalation of cases during 2005 and 2006, as well as the deaths in infected neonates. Our awareness of the disease (and the real possibility of there being a worldwide problem) was increased by the reports of primary infections in Italy during the summer of 2007. Nonetheless, more needs to be done to educate the public about the risks associated with re-emergent viruses such as CHIKV. Clearly, a virus capable of infecting an estimated 7.5 million people over a 5-year period, resulting in chronic arthralgia in ~30% of these individuals, deserves more attention. Private and public funding organizations have helped to raise awareness for global health issues such as HIV infection, malaria and tuberculosis, but this unfortunately represents only a proverbial 'small bite' out of a major problem.

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Matthew L. Albert's homepage: http://www.pasteur.fr/ip/easysite/go/03b-000010-002/dendritic-cell-immunobiology
WHO guidelines on the Clinical Management of Chikungunya: http://www.searo.who.int/LinkFiles/Publication_guidelines_on_cli_mgmt_chikungunya_fvr-(cd-180).pdf

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