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REVIEW ARTICLE

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Pathogenicity and virulence of chikungunya virus

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ABSTRACT

Chikungunya virus (CHIKV) is a mosquito-transmitted, RNA virus that causes an often-severe musculoskeletal illness characterized by fever, joint pain, and a range of debilitating symptoms. The virus has re-emerged as a global health threat in recent decades, spreading from its origin in Africa across Asia and the Americas, leading to widespread outbreaks impacting millions of people. Despite more than 50 years of research into the pathogenesis of CHIKV, there is still no curative treatment available. Current management of CHIKV infections primarily involves providing supportive care to alleviate symptoms and improve the patient's quality of life. Given the ongoing threat of CHIKV, there is an urgent need to better understand its pathogenesis. This understanding is crucial for deciphering the mechanisms underlying the disease and for developing effective strategies for both prevention and management. This review aims to provide a comprehensive overview of CHIKV and its pathogenesis, shedding light on the complex interactions of viral genetics, host factors, immune responses, and vector-related factors. By exploring these intricate connections, the review seeks to contribute to the knowledge base surrounding CHIKV, offering insights that may ultimately lead to more effective prevention and management strategies for this re-emerging global health threat.

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1. CHIKV distribution and vectors

1.1. The virus and its spread for world domination

Chikungunya virus (CHIKV) is a mosquito-transmitted virus of the *Togaviridae* family. This single-stranded, positive-sense RNA virus (ssRNA) is known to cause widespread outbreaks of arthritogenic illness globally, resulting in a significant public health burden. First isolated in Tanzania in 1952 from a febrile patient [1], CHIKV is believed to have evolved in Africa at least 300–500 years ago [2]. After its initial isolation, confirmation soon emerged of CHIKV infection in Asia in the mid-1950s [3] with the first evidence of clinical infection from a patient in India in 1954 [4] and the first laboratory-confirmed outbreak in Thailand in 1958 [5]. Between the 1960s-1980s, reports continued of sporadic outbreaks of CHIKV in Africa and Asia, as reviewed by Zeller et al. [6]. Evidence of CHIKV circulation and disease then declined, and it was not until 2004 that the virus significantly re-emerged.

In 2004, an outbreak of CHIKV occurred in Kenya before spreading in 2005–2006 to the islands of the Indian Ocean, including Comoros, La Réunion, Mauritius, and Mayotte [7]. The outbreak that occurred in La Réunion between 2005 and 2006 is still possibly the most famous to date, owing to the high incidence with approximately one-third of the population affected [8] and complex clinical presentations, including fatal cases [9–11]. From 2005 onwards, CHIKV rapidly became a global virus, with outbreaks in Asia and cases in Europe, followed by the spread of the virus to the Americas in 2013. Between 2005 and 2006, India reported a widespread outbreak of CHIKV with approximately 1.3 million cases [12]. Since 2006, outbreaks have emerged throughout Asia, including Sri Lanka (2006), Malaysia (2006–2007), Thailand and Singapore (2008–2009), China (2010), and Cambodia (2011).

In 2007, the CHIKV reached Europe with cases of local transmission in Italy [13]. The virus was believed to have been introduced by a returned traveller from India, resulting in over 200 cases of local transmission. In 2010, a similar outbreak occurred in the south of France again from an introduced case and local transmission attributed to *Ae. albopictus* mosquitoes. Since this time, smaller outbreaks have been reported periodically throughout Southern Europe.

The virus was first detected in the Pacific in 2011 with the autochthonous transmission of CHIKV in

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New Caledonia [14]; however, case numbers were initially low and public health measures were rapidly employed to limit the virus spread. In 2012, Papua New Guinea began to record an increase in febrile illnesses with subsequent arthralgia symptoms, and soon after, a CHIKV outbreak was reported [15]. By 2013, CHIKV was taking hold in the Pacific region with an outbreak in New Caledonia and Yap State, closely followed by outbreaks in Tonga, American Samoa, the Independent States of Samoa, and Tokelau. In 2014, an outbreak was reported in French Polynesia involving 25% of the population, followed by the Cook Islands in 2015. Whereas the spread of CHIKV in the Pacific appeared to follow a linear trend, phylogenetic analysis of the virus shows that both the ESCA and Asian lineages were circulating in the region.

By 2013, CHIKV had reached the Americas with its first detection in the Caribbean. From here, CHIKV rapidly moved throughout the Americas resulting in over 1 million cases in the first year. In 2014, CHIKV reached the USA with local transmission occurring in Florida, Texas, Puerto Rico, and the U.S. Virgin Islands [16,17]. The virus has continued to circulate in endemic and epidemic patterns in the Americas and now circulates in over 50 countries and territories in the region, with more than 75% of the world population estimated to be at risk of CHIKV infection [18].

1.2. Recent trends and emerging epidemics

In the past few years, CHIKV has continued to circulate across the globe. There have been reports of outbreaks in Pakistan and India 2016–2017, France 2017, Brazil 2014–2017, Republic of Congo 2019, Ethiopia 2019, and continued circulation in South America. In 2023, there are ongoing reports of CHIKV cases in South America with cases in Bolivia, Peru, Venezuela, new local transmission in Argentina and Uruguay [19] and Paraguay reporting its largest outbreak to date [20].

1.3. Mosquito vectors

CHIKV is a global public health threat transmitted primarily by the widespread *Aedes (Ae.) aegypti* and *Ae. albopictus* mosquitoes [21]. *Ae. aegypti*, which also transmits other significant arboviruses including dengue virus (DENV), Zika virus, and yellow fever virus [22], is prevalent in tropical and subtropical regions around the world. *Ae. aegypti* commonly dwells as an urban, indoor mosquito primarily feeding on humans [23] and is largely restricted to warmer climates due to the inability to overwinter [24,25]. On the other hand, the Asian Tiger mosquito *Ae. albopictus*, which also transmits multiple arboviruses, can be found in subtropical regions as well as colder, temperate regions and can overwinter as eggs and adults in colder climates [25]. As such, *Ae. albopictus* has a larger geographic range and tends to live outdoors and further from humans, feeding on other animals in nature. Interestingly, CHIKV has also been detected in other *Aedes species* such as *Ae. furcifer*, *Ae. taylori*, *Ae. luteocephalus*, *Ae. africanus*, *Ae. dalzieli* and *Ae. neoafricanus* and also in *Anopheles coustani*, *Anopheles rufipes,* and *Culex ethiopicus* [26]. However, the role and significance of these mosquitoes in the transmission of CHIKV is uncertain.

1.4. Vector-related factors in CHIKV transmission

There are several factors that may influence CHIKV vector transmission dynamics and epidemic potential, including the mosquito itself, the environment, and the virus [27]. Vector competence, or the ability of a vector to transmit a pathogen, is critical for CHIKV spread. Laboratory studies have shown that different geographic populations of mosquitoes can transmit CHIKV differently, suggesting geographic and genetic roles for transmission dynamics [28–32]. In addition to mosquito genetics and location, temperature is an important driver of CHIKV vector transmission [24,33,34]. It has been found that changes in temperature can significantly influence CHIKV vectorial capacity and transmission in the lab [34]. Moreover, given that *Ae. aegypti* is restricted to warmer climates, increases in global temperatures could cause these vectors to migrate to naïve populations, spreading CHIKV and other arboviral diseases. Using comprehensive databases, environmental variables, and sophisticated modelling approaches [35,36], recent studies predicted a migration towards northern and southern regions that could be explained by human activities causing climate warming, thus creating new favourable environments for *Aedes spp* [37]. This phenomenon has already begun to occur in Europe, where the first autochthonous cases of flavivirus infections have occurred in recent years [38–40]. The distribution of arthropod vectors and CHIKV is influenced by a complex interplay of environmental factors, human activities, and the presence of susceptible hosts that, when aligned, typically lead to disease outbreaks.

Another critical factor is the mosquito microbiota, which resides in the digestive tract and influences virus biology after a blood meal [41] by regulating the immune response [42–44]. Notably, the presence of the intracellular bacterium Wolbachia (Wb), found in approximately 60% of insects, serves as a well-known

example of such influence [45]. Interestingly, it naturally infects *Ae. albopictus* but not *Ae. aegypti* [46]. Consequently, scientists have artificially introduced Wb into *Ae. aegypti* [46,47]. Wb is known to interfere with the biology of various pathogens, including parasites and viruses like CHIKV [48,49] and modulates the immune response of *Ae. aegypti* by increasing the expression of cellular factors such as thio-ester-containing proteins (TEPs), C-type lectins, defensin, diptericin, Glycosaminoglycan-Binding Protein B1 (GNBPB1), Serine Protease Z1A (PZ1A), cactus, and cecropin [50]. These cellular factors may play a crucial role in neutralizing CHIKV proliferation within the mosquito [51]. For instance, TEPs are crucial for countering pathogen proliferation in mosquitoes, analogous to complement C3 in mammals [52–54]. While the role of TEPs during CHIKV infection has not been studied yet, their significance in the modulation of the immune response in *Ae. aegypti* has been demonstrated in DENV infection [55]. Silencing TEP1 in mosquitoes increased viral RNA (vRNA), viral proteins, and infectious particles, indicating that TEP1 counters DENV replication by modulating the innate immune response.

2. CHIKV and Viral replication

2.1. Genome structure and organization

CHIKV, like other members of the *Togaviridae* family, contains a single-strand, positive-sense RNA genome (~12 kb) that has a 5' N7-methylguanylated cap and a polyadenylated 3' tail [56,57] (Figure 1a). The genome contains 5' and 3' untranslated regions (UTRs), which flank two open reading frames (ORFs) coding for the non-structural proteins (nsP) and the structural proteins (sP) [57–59]. The 5' ORF encodes four nonstructural proteins (nsP1, nsP2, nsP3, and nsP4) that are pivotal for genome synthesis and other replicative functions and are typically translated as a polyprotein of nsP1-3 or nsP1-4 via read-through of an opal stop

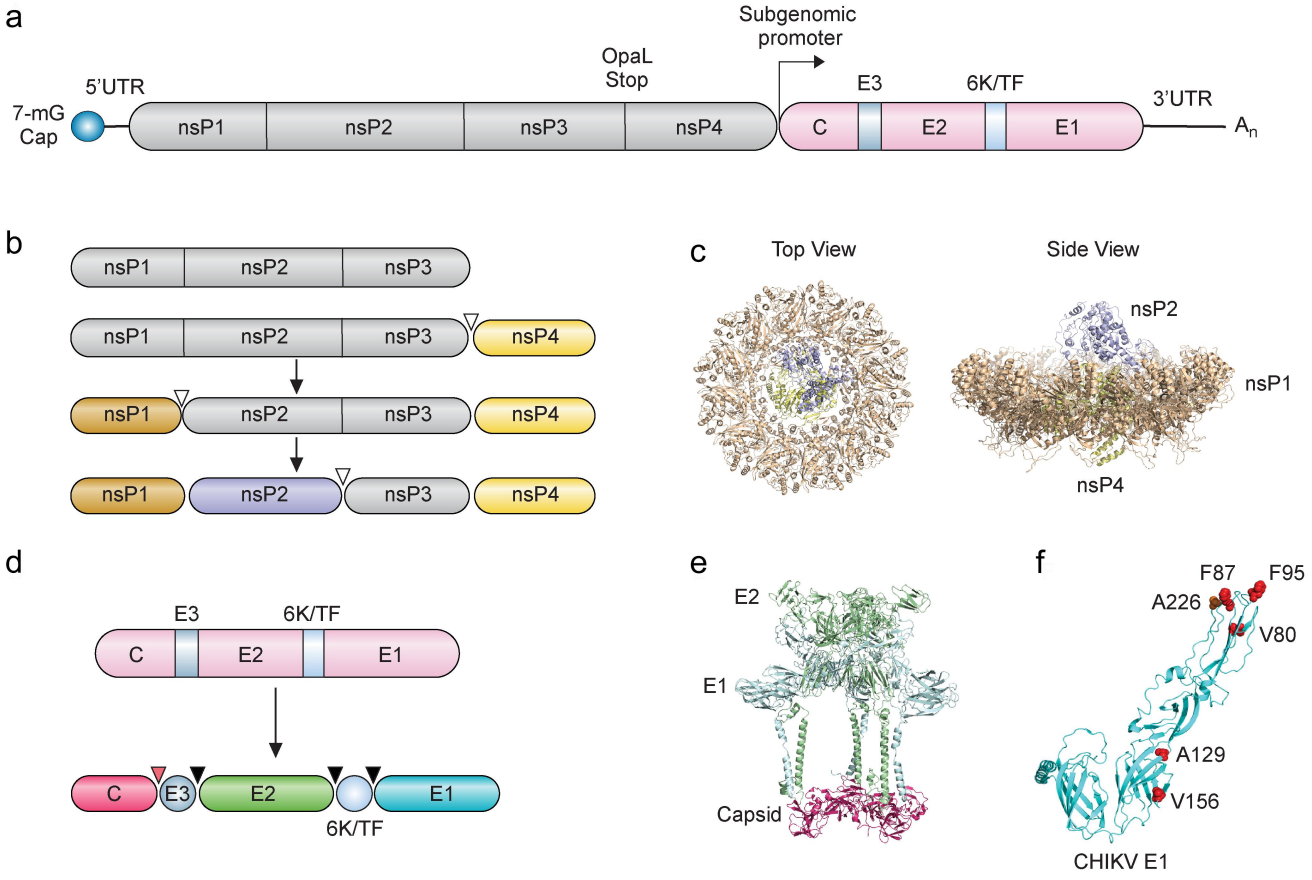

Figure 1. CHIKV genome organization, protein processing, and protein complex structure.

(a) Schematic of CHIKV genome. (b) Nonstructural protein polyprotein processing. Open arrowheads signify nsP2 cleavage sites. (c). CHIKV replication complex structure from top and side view (PDB ID: 7DOP). nsP1 is in tan, nsP2 is in purple, and nsP4 is in yellow. (d). Structural protein polyprotein processing. Red arrowhead signifies capsid autocleavage, and black arrowheads signify host peptidase cleavage. (e). CHIKV VLP glycoprotein complex with capsid (PDB ID: 6nk5), and (f). CHIKV E1 crystal structure (PDB ID: 6nk5). E1 residues in red correspond to residues contributing to transmission and/or virulence. (PMID: 24922573, 31291581, 35416719, 34935436)

codon between nsP3 and nsP4 (Figure 1a) [57,59–61]. The 3' ORF, which is translated from a subgenomic RNA (sgRNA) that is transcribed from the negative-sense antigenome, encodes for a polyprotein comprised of the viral sPs (Capsid, E1, E2, E3, 6K, and TF), which constitute the actual virus particle and are responsible for virion assembly, budding, and the process of entering host cells (Figure 1a) [57,62]. Both polyproteins are processed by viral or host proteases during viral replication with specific temporal and spatial patterns (Figure 1b,d) [56,63]. The CHIKV virion is small in diameter (~70 nm) and is comprised of an icosahedral nucleocapsid shell that encapsulates the viral RNA genome and is surrounded by a lipid bilayer envelope (Figure 1c) [62]. Trimeric spikes formed by heterodimers of the E1 and E2 glycoproteins are embedded within the viral membrane and form an outer icosahedral protein lattice (Figure 1e) [57,62,64].

2.2. Viral entry

CHIKV displays a broad tropism, infecting many different cell types within different species, and the current model of CHIKV entry suggests binding to a variety of entry factors in complex and multifaceted mechanisms that are likely to be distinctive in different cell types [65–67]. The E1 and E2 viral glycoproteins mediate cell entry, with E2 facilitating virus attachment and E1 functioning as a class II fusion glycoprotein [68]. Entry of CHIKV into mammalian cells is initiated by interactions with cell-surface glycosaminoglycans (GAGs) (Figure 2) [69–72] present in high abundance on most cells [73,74]. CHIKV binds to heparan sulphate (HS), chondroitin sulphate (CS), and dermatan sulphate (DS), with a higher affinity for HS [70]. CHIKV binding to GAGs mediates viral adhesion to the cell surface and enhances infection [69,70,72,75], but the level of dependence on GAGs for efficient infection is likely to be cell-type dependent based on the presence and expression levels of entry factors utilized in different cells [70]. Various entry factors have been implicated in CHIKV cell entry, including the CD147 protein complex [76], C-type calcium-dependent lectins DC-SIGN [77] and L-SIGN [67], Matrix remodelling-associated protein 8 (MXRA8) [78], prohibitin [79], and the phosphatidylserine-dependent receptors, TIM-1, and Axl [67,80]. E2 is thought to be the main attachment protein that binds to entry receptors, but E1 has also been implicated in binding to both attachment factors [81] and entry receptors [82,83].

To date, MXRA8 has been the most studied entry receptor for CHIKV, and also serves as an entry receptor for Mayaro, o'nyong'nyong (ONNV), Ross River, and Semliki Forest viruses [78]. MXRA8 is a single pass type I transmembrane adhesion molecule with two IgG-like domains that displays structural homology to other viral receptors like Coxsackie and adenovirus receptor (CAR) and Junctional-adhesion molecule A (JAM-A) [82,83]. MXRA8 is expressed by many target cell types infected by CHIKV, and knockout of MXRA8 in permissive cells reduces infection *in vitro* and *in vivo* [84]. Direct binding between MXRA8 and CHIKV has been observed [78] and cryo-EM studies revealed a complex interaction involving both domains of MXRA8 contacting numerous residues in the "canyon" between adjacent E1/E2 trimer receptors [82,83]. After the virus binds to surface MXRA8, they are co-internalized into cells and can co-traffic until fusion occurs [85], further establishing MXRA8 as a bona fide entry receptor for CHIKV. However, MXRA8 is not expressed on all target cell types [78,86] and when *Mxra8* KO mice are infected with CHIKV, viral replication is reduced but not abrogated [84], indicating that CHIKV binds to additional receptors *in vivo*. Entry factors for mosquitoes have been more difficult to identify, with only the ATP synthase ß subunit [87] and Heat Shock Chaperone 70 [88] reported as host factors that facilitate CHIKV entry into mosquito cells in culture.

Receptor-mediated CHIKV endocytosis is thought to be facilitated mainly by clathrin [85,89–91], although other entry routes like micropinocytosis have been observed for CHIKV in some cell types [92]. Entry through micropinocytosis may occur through the uptake of apoptotic bodies harbouring CHIKV particles from neighbouring infected cells [93]. Although CHIKV can enter cells through multiple endocytic pathways, viral particles are delivered to early endosomes, marked by Rab5, where CHIKV fusion mainly occurs [90,91]. Endosomal acidification is required for membrane fusion as low pH causes destabilization and dissociation of the E2-E1 heterodimer, leading to the exposure and insertion of the E1 fusion loop into the target membrane, trimerization of E1, and fusion of the viral and cell membranes [94,95]. Compounding the complexity of CHIKV entry is the observations that CHIKV strains vary in their dependence on some entry factors [70,78], display different pH thresholds [95] and cholesterol requirements [96], utilize entry pathways differently [91,92], and in their ability to infect various cell lines [92,97–99]. Thus, the complex mechanisms that mediate CHIKV entry into different cell types have yet to be completely unravelled.

2.3. Establishing genome replication

The replication cycle of CHIKV in mammalian cells has not yet been fully elucidated, but recent work has unveiled new and exciting mechanistic details into the

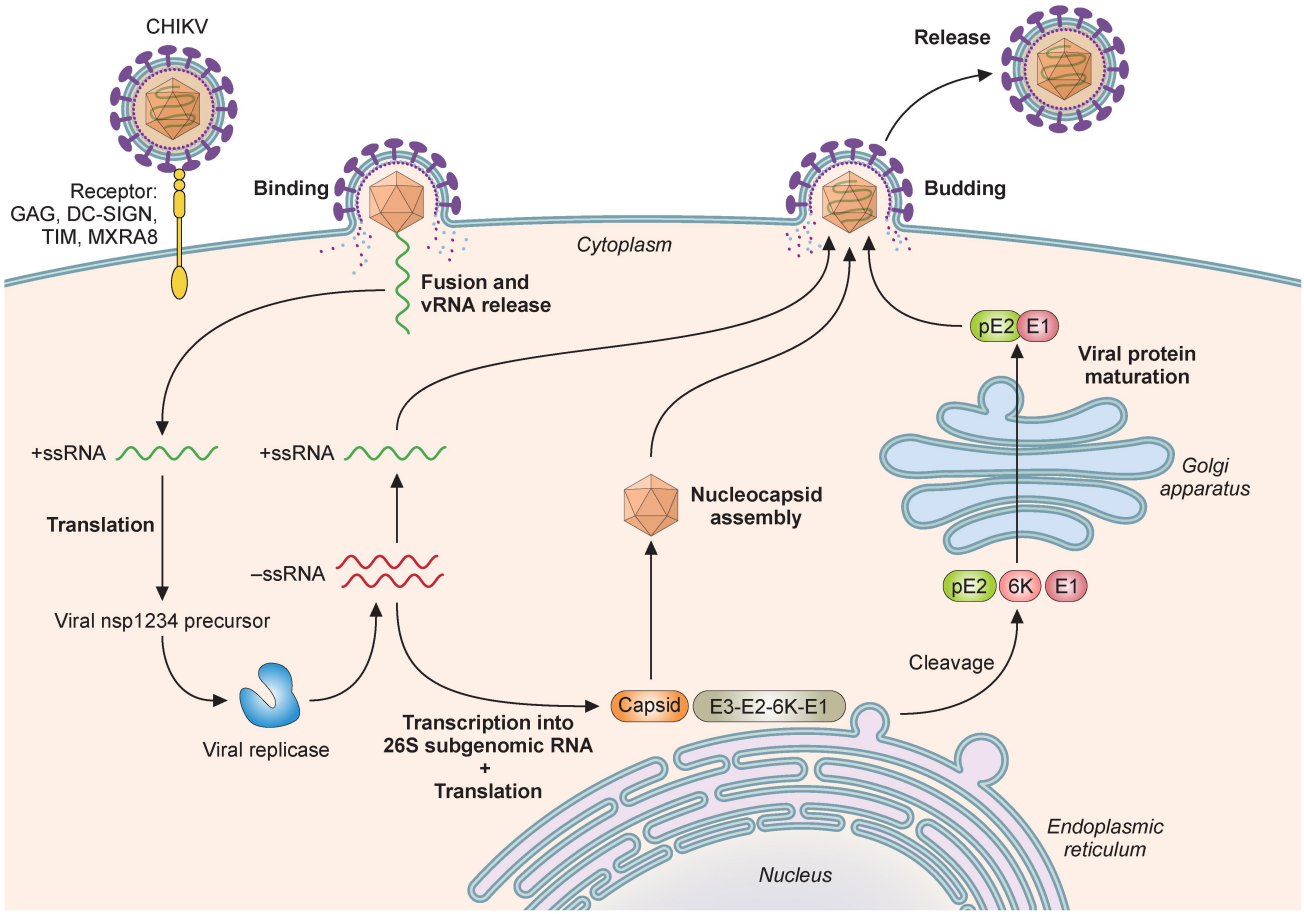

Figure 2. Chikungunya virus replication cycle.

CHIKV E2 glycoprotein binds to host membrane factors (e.g. GAGs, MXRA8, DC-SIGN, and TIM) and enters via a clathrin-mediated endocytosis pathway. Following fusion of the endosomal and viral membranes, the nucleocapsid is released in the cytoplasm, exposing the viral genomic RNA to the host translation machinery. Translation of the non-structural polyprotein P1234 takes place, and cleavage of the nsP2 polyprotein by nsP2 yields an active viral replicase, which is responsible for the synthesis of the -ssRNA antigenome, the 26S subgenomic ssRNA, and genomic ssRNA. The 26S subgenomic RNA is translated into a polyprotein C-pE2-6K-E1 at the rough endoplasmic reticulum membrane. The C protein cleaves itself from the polyprotein and assembles with the newly synthesized +ssRNA to form the nucleocapsid. The pE2-6K-E1 polyprotein is cleaved into pE2, 6K, and E1, which undergo maturation through Golgi apparatus. Matured E1-E2 heterodimers form virus budding microdomains at the PM, allowing budding of virions containing the nucleocapsid. Newly assembled virions are released to the extracellular environment.

viral replicase and additional functions of viral proteins. Fusion of the viral and early endocytic membranes releases the nucleocapsid (NC) into the cytoplasm (Figure 2) [90], where interaction between a ribosome binding site in the N-terminus of Capsid and the host 60S ribosomal subunits triggers rapid disassembly of the NC and delivery of the genome into the cytosol [100]. As the viral genome mimics host mRNA, the nsP polyprotein is directly translated and cleaved into individual proteins by the papain-like protease activity of nsP2 [101]. However, the precise spatial and temporal orchestration of nsP polyprotein processing is critical for the viral replicase to function in a stepwise manner [102]. Most CHIKV strains contain a highly conserved opal stop codon after nsP3, allowing for translation of nsP1-2-3 with low-frequency readthrough to produce lower levels of nsP4 [61,103,104]. In some strains, the opal codon is replaced by an arginine residue, and the polyprotein is translated as nsP-1-2-3-4 and protease activity of nsP2 rapidly releases nsP4 from *p*-1-2-3 [102]. Palmitoylated residues in a dodecameric ring of nsP1 direct the viral replicase to cholesterol-rich microdomains (termed lipid rafts) of the plasma membrane (PM) inner leaflet [105–108], where negative-strand synthesis is initiated by nsP1-2-3, nsP4, and host proteins [102]. nsP1 initiates negative-strand synthesis through guanyltransferase and guanine-7-methyltransferase activities that mediate RNA capping. The NTPase, RNA 5'triphosphatase, and helicase activities of nsP2 assist the nsP4 RNA-dependent RNA

polymerase (RdRP) to generate full-length antigenomic RNA [102]. A subgenomic promoter in the antigenome drives the transcription of the CHIKV sgRNA encoding the structural proteins required for virion assembly (Figure 2). The subgenomic RNA, which is also capped and polyadenylated, is also translated as a polyprotein.

2.4. Virus replication organelles

Generation of the negative-strand antigenome facilitates the formation of viral replication organelles, termed spherules, which are extracellular, bulb-shaped organelles (~50–80 nm in diameter) housing a single copy of the antigenome in duplex with the positive-sense viral genome [109]. These dsRNA intermediates generated during genomic replication are shielded inside spherules from innate immune detection systems [110]. Spherules are conjoined with the cytoplasm by a stable, open neck that is maintained by a crown-like complex of proteins (Figure 1b) positioned at the base of the neck and comprised of viral nsPs and host proteins [109,111]. The recently determined cryoET ultrastructure of the core viral replicase complex revealed nsP2 and nsP4 to be localized at the centre of a dodecameric nsP1 ring that lines the neck, while nsP3 is thought to be present in an outer cytoplasmic ring in complex with host components [111]. The host proteins that promote CHIKV RNA synthesis are largely unknown but are likely recruited by nsP3 through its hypervariable domain, a largely disordered, phosphorylated domain whose sequence varies greatly between alphavirus members [112,113]. Increased production of nsPs results in nsP2-mediated cleavage of nsP1 from nsP2-3 [114], which directs a switch to transcription of positive-strand vRNAs that are thought to be spooled out of the neck of the spherule into the cytoplasm [105]. The cleavage between nsP2-3 facilitates the transcription of the sgRNA encoding the viral sPs [115], which also mimics host mRNA. Multiple spherules can be internalized into endocytic vesicles and form large vacuolar structures (up to 2,000 nm), called cytopathic vacuoles type-1 (CPV-1).

2.5. Virion assembly and release

Once the structural polyproteins are translated from the sgRNA, the autoproteolytic activity of Capsid releases it from the rest of the sP polyprotein into the cytosol (Figure 2). Capsid interacts with specific sequences within newly synthesized genomic RNA promoting the oligomerization of capsid molecules and genome encapsidation [116,117]. The remaining polyprotein (E3-E2-6K/TF-E1) is directed to the host endoplasmic reticulum (ER) membrane due to the signal sequence at the N-terminus of E3 [118]. Ribosomal frameshifting in the 6K sequence produces TF, whose 63 N-terminal amino acids are identical to that of 6K but have a unique C-terminus, followed by a stop codon preventing translation of E1 [119,120]. ER peptidases cleave the structural polyproteins, releasing the accessory proteins 6K and TF from the glycoproteins [121]. After processing in the ER, pE2 (uncleaved E3-E2) and E1 remain associated as they are transported through the secretory pathway, undergoing conformational changes and post-translational modifications (N-linked glycosylation and palmitoylation) to form an immature, non-fusogenic spike complex [122]. Virally induced type 2 cytopathic vacuoles (CPV-2) form late in infection from the trans-Golgi network near the PM and contain p62-E1 trimer-like structures, sometimes observed in helical tubular arrays [123]. Due to critical interactions between the cytoplasmic tail of E2 and Capsid, newly formed NCs are located on the cytoplasmic face of CPV-2s [123]. CPV-2s are likely an intermediate assembly point and are thought to be trafficked to the PM to deliver NCs and glycoproteins for the assembly of virions [124].

E3 is thought to act as a chaperone during trafficking to the PM, aiding in spike formation and protection of the E1 fusion loop, until it is cleaved from E2 late in the secretory pathway by furin-like proteases (Figure 2) [125]. Cleavage of E3 from E2 allows for the formation of mature fusion-competent E1/E2 spikes, due to small structural rearrangements in the viral glycoprotein domains and their cytoplasmic tails [126]. These changes in the spike cause in turn an expansion of the nucleocapsid, revealing the capsid ribosome-binding site and priming the NC for disassembly in the next infected cell [126]. E3 remains associated with E2 in low pH environments (~5.5–6.2) of the Golgi and secretory vesicles to prevent premature triggering of the spike complex. E3 is often released in the neutral pH of the extracellular space [127–130]; however, cleaved E3 can remain associated with virions, which may influence receptor usage and cell entry [82,131–133].

Although the functions of 6K and TF in assembly are not fully understood, their roles in replication and infection are likely disparate from each other [134]. 6K and TF have an identical N-terminus but unique C-termini, resulting from ribosomal frameshifting, and both are nonessential in tissue culture [119,135,136]. 6K is a virus-derived ion channel or viroporin that resides mainly at interior cellular membranes and is thought to interact with E2 [137,138] to aid in glycoprotein maturation and virus budding

[137,139,140]. Palmitoylated TF, which aids its membrane localization and incorporation into virions at a higher frequency than the more abundant 6K protein, also plays a role in virion assembly [119,120,134,141].

CHIKV egress occurs at the PM of the cell body and from virus-induced extensions of the PM [142–146]. Budding may occur within specific regions of the PM, which are areas of concentrated structural proteins and budding viruses [143]. Host proteins are thought to be mostly excluded from these budding sites and are not readily found in the rigidly structured virions [122,143]. Assembled glycoprotein spikes form vertical links across the lipid bilayer by insertion of the intracellular tail of each E2 into a hydrophobic pocket of the C-terminus of a corresponding capsid, yielding two icosahedral protein layers of NC and trimeric spikes [147,148]. Multiple intermediates in the budding process of CHIKV particles have been observed and reveal a stepwise formation of mature particles [64]. Immature non-icosahedral NCs are thought to serve as an early, spherical scaffold that initiates lateral interactions between trimeric spikes into icosahedral shells, which in turn facilitate the continued assembly and maturation of the NCs [64,143], until fully assembled virus particles are released into the extracellular space. CHIKV can also be transmitted to nearby uninfected cells through virally induced intercellular long extensions (ILEs; > 10 uM) [142,143,145,146,149,150], which display active viral budding of nascent, infectious virus particles along the length of the extension and concentrated at the tip [142,143].

3. Infection, Clinical Disease, and Diagnostics

3.1. Overview of infection

The early steps of CHIKV infection in humans are still poorly understood, but current evidence suggests a possible succession of events based on current knowledge of CHIKV and other arboviral infections. During a blood meal by an infected mosquito, CHIKV can be deposited directly into the bloodstream or within dermal tissue, along with mosquito saliva, which has components that dampen the interferon signalling pathway and enhance viral replication (Figure 3) [151–154]. In the dermis, CHIKV replicates in dermal fibroblasts, which act as sites of viral amplification [89,152,155–159]. Other possible dermal resident cells that may be infected by CHIKV include dendritic cells [160], macrophages [161], endothelial and epithelial cells

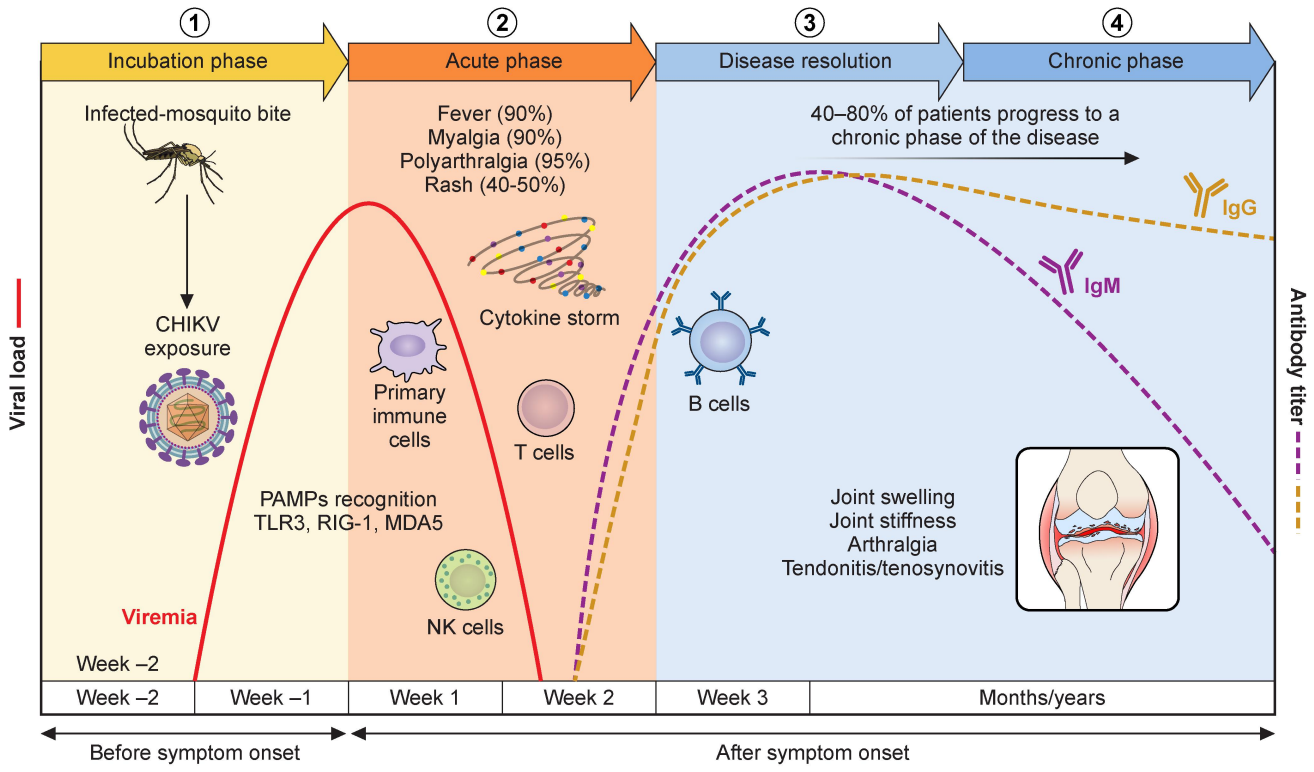

Figure 3. Timeline of chikungunya virus pathogenesis.

Schematic of the timeline of CHIKV-induced disease divided into four phases: incubation, acute, disease, resolution, and chronic illness. Typical viral load (pink line), symptoms, cytokine storm, involved immune cells, and antibody titres (orange and purple dotted lines) are depicted along the infection timeline.

[89], keratinocytes [97,154], and melanocytes [162]. Through binding, infection, or uptake by antigen-presenting cells in the skin, it is thought that CHIKV travels through the lymphatics into the bloodstream [163]. From the bloodstream, CHIKV disseminates systemically throughout the body to secondary sites of replication, including tissues where disease symptoms are prominent (connective tissue, muscle, peripheral joints, and tendons; discussed in further detail below) [155,157,161,164–167]. As humans are amplifying hosts of CHIKV, infection leads to high viraemia [168], which is required for subsequent infection of and transmission from a blood-feeding mosquito.

3.2. Acute CHIKV disease

Clinical presentation of CHIKV-induced disease (CHIKVD) has a symptomology that overlaps much of that known for DENV infection, including fever and joint and muscle pain, and as such there have been suggestions that CHIKVD was widespread in the 19th century [169]. Following an incubation period of approximately 1–12 days [170,171], patients usually report acute symptoms such as high fever (often exceeding 102°F/39°C), joint pain (arthralgia), headache, fatigue, and muscle pain (myalgia) (Figure 3), all of which can be both debilitating and distressing [172–175]. Joint pain, the hallmark feature of CHIKVD, is frequently bilateral and symmetric, affecting corresponding joints on both sides of the body including hands, feet, knees, and wrists [176]. CHIKV displays a tropism for synovial tissues, inciting an immune response that results in joint inflammation and intense pain [177]. The severity and duration of symptoms can vary significantly among individuals. Simultaneously with muscle pain, headache, and fatigue, some individuals experience gastrointestinal symptoms [176]. In addition, CHIKV can cause neurologic symptoms in complicated cases such as encephalitis, optic neuropathy, neuroretinitis, Guillain – Barré syndrome [178], and the most common being the meningoencephalitis [179,180]. Finally, there is growing evidence that CHIKV-infection can cause cardiovascular symptoms, including arrhythmias, myocarditis, and cardiomyopathy [181].

Finally, even though CHIKV has a low rate of mortality and therefore is typically considered non-fatal [182], deaths do occur often in cases with comorbidities or with more rare but severe symptoms of CHIKV disease. CHIKV-associated neurological diseases start between 1 and 3 weeks post-infection and are the major cause of death related to CHIKV [11,178,183]. In 2023, the European Centre for Disease Prevention and Control reported approximately 320,000 CHIKV cases in 20 countries and more than 340 deaths worldwide as of 23 August 2023 [184].

3.3. Chronic CHIKV disease

While most people recover fully from CHIKV, others, particularly those with underlying health conditions, may suffer from prolonged joint pain and other symptoms, leading to chronic discomfort and reduced functionality (Figure 3) [185]. In fact, 40%–80% of patients progress to a chronic phase of the disease, with symptoms lasting more than three months (Figure 3) [186,187]. Joint pain and inflammation can closely resemble rheumatoid arthritis (RA) in a clinical and molecular aspect [188], leading to misdiagnoses and challenges in distinguishing the two conditions. Moreover, chronic disease can have a profound and enduring impact on both affected individuals and the communities they reside in, often resulting in prolonged pain and disability, reduced workforce productivity, and increased healthcare expenses [189].

3.4. CHIKV Diagnostics

Accurate and timely diagnosis of CHIKV infection is crucial for patient management, epidemiological surveillance, and outbreak control. Several diagnostic methods and tools are available to detect CHIKV and differentiate it from other similar diseases (Figure 4) [190]. Clinical evaluation typically begins with assessing the patient's symptoms, which often include a sudden onset of fever, joint pain, rash, and other flu-like symptoms. While clinical signs are non-specific and can overlap with other mosquito-borne illnesses with similar clinical presentations, such as dengue fever, Zika virus infection, and other arboviral diseases [191], they can provide initial clues for CHIKV infection.

Whereas clinical guidelines on the approved methods to conclusively diagnose CHIKV infection vary across the globe, the gold standard is the detection of CHIKV nucleic acid through reverse-transcription polymerase chain reaction (RT-PCR) (Figure 4) [192,193]. Although RT-PCR can be an expensive detection method for developing countries [194], this method detects the presence of the virus in patient samples, such as blood, serum, or plasma and therefore is conclusive of CHIKV infection. RT-PCR is highly sensitive and specific and can identify CHIKV during the acute phase of infection (collected within 7 days of the initial appearance of symptoms), during which the viral load can reach levels exceeding 1×10^{11} copies/ml of serum [195]. Real-time RT-PCR provides

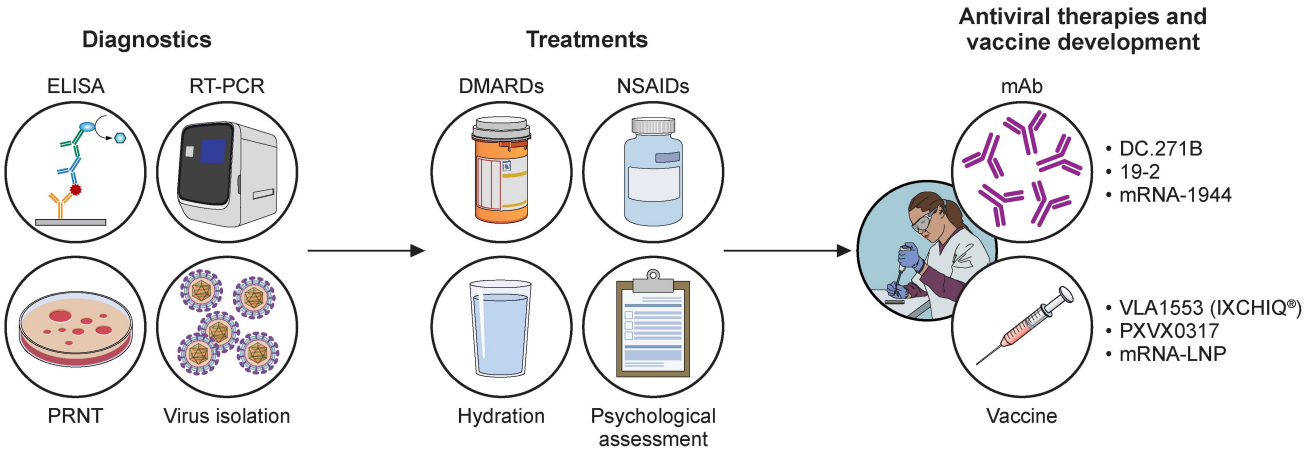

Figure 4. Timeline of chikungunya disease management.

Schematic depicting the different strategies to diagnose CHIKV infection (ELISA, RT-PCR, PRNT, virus isolation), the different treatments currently available (DMARDs, NSAIDs, hydration, and psychological assessment), and the current new and promising therapeutical strategies (mAb and vaccine).

quantitative results and allows for viral load determination, aiding in disease monitoring and outbreak investigations [196,197]. In 2007, the US Centers for Disease Control and Prevention (CDC) developed a qRT-PCR assay that was systematically used for CHIKV detection [198]. However, this qRT-PCR was based on CHIKV strains before the 2014 American pandemic and may not include more recent strains, potentially leading to a decrease in sensitivity. Edwards and colleagues challenged this method in 2017 with recent CHIKV strains from Central and South America [199]. They optimized the method to specifically detect a highly conserved domain segment of E1, allowing for the detection of all CHIKV lineages. In addition, in 2016 the CDC developed a trioplex real-time RT-PCR assay that detects CHIKV, DENV, and ZIKV vRNA in human sera or cerebrospinal fluid [200]. This method, challenged and optimized by Santiago et al. in 2018 [201], showed a high sensitivity for all three viruses. Remarkably, this assay was extensively used during the ZIKV epidemic in South America in 2016–2017, allowing the detection of more than 30,000 cases [202]. While this assay is used for different clinical specimens, the FDA has only approved its use for the detection of ZIKV in amniotic and cerebrospinal fluids [201].

In incidences where the virus itself cannot be detected; infection can also be diagnosed by serological diagnosis either by paired IgM/IgG serology or by demonstrating a fourfold rise in antibody titres in acute versus convalescent sera (usually separated from 7 to 14 days). The commonly used method for antibody detection is the enzyme-linked immunosorbent assay (ELISA), which can detect antibodies (IgM and IgG) against CHIKV in serum or plasma samples (Figure 4) [203]. Recent studies have confirmed its ability to detect CHIKV-specific antibodies in patient sera, even in the case of previously negative tests in the acute phase [204]. One of the strengths of ELISA is its utility in early diagnosis but also several months after the initial infection. Recent advancements have improved the performance of ELISA assays for CHIKV detection, including the development of multiplex ELISA platforms that can simultaneously detect antibodies to multiple arboviruses, including CHIKV, Dengue, and Zika [205–207]. Additionally, novel ELISA formats, such as the microsphere-based assay, have shown promise in enhancing sensitivity and specificity in arboviruses [208–210].

In some circumstances, CHIKV infection is diagnosed following virus isolation (Figure 4). This involves attempting to grow CHIKV from patient samples, such as blood or serum, in a laboratory setting. However, due to its classification as a risk group 3 pathogen, its culture is challenging as it requires biosafety level 3 (BSL-3) precautions [89,211,212]. Although less commonly used due to its complexity and time-consuming nature, it provides a definitive diagnosis and allows for further research and strain characterization [213].

The plaque reduction neutralization test (PRNT) is a method used to measure the level of neutralizing antibodies in a patient's sample and has been used in identifying previous viral infections, assessing vaccine effectiveness, and studying the prevalence of antibodies in a population (Figure 4) [214]. It involves exposing a sample of the patient's serum to infectious CHIKV virions and measuring the highest serum dilution that can still neutralize the virus [215,216].

4. Pathogenesis

The pathogenesis of CHIKVD is complex and involves a combination of viral factors, host immune responses, and interactions with specific cellular factors and pathways. Adding to the complexity of the CHIKVD process, the host immune response is required to clear infection, but also contributes to the pathogenesis of CHIKV-induced disease. This section will discuss the pathogenesis of CHIKV, how the host responds to infection, and the different mechanisms by which CHIKV evades the immune response.

4.1. Innate immune responses: Friend and foe?

Innate immunity is essential for combatting CHIKV infections by early detection of CHIKV infection and priming the adaptive immune response, which aids in clearance of the virus but also drives inflammation. As an RNA virus, CHIKV is recognized as "non-self" by host pattern recognition receptors (PRRs), including like toll-like receptors TLRs (Figure 5). The absence of TLR3, which recognizes double-stranded vRNA, exacerbates the severity of CHIKV infection in human and mouse fibroblasts, showing its important role against CHIKV [217]. Moreover, analysis of TLR3 by SNP genotyping in CHIKV-patients revealed a correlation between SNP TLR3–rs6552950 and the severity of the disease [217]. Additionally, TLR4, which senses bacterial lipopolysaccharides or damage-associated molecular patterns [218], plays a crucial role in facilitating the attachment and entry of CHIKV in host macrophages, contributing to infection and modulation of pro-inflammatory responses [219]. Inhibition of TLR4 using TAK-242 reduces viral load, CHIKV-E2

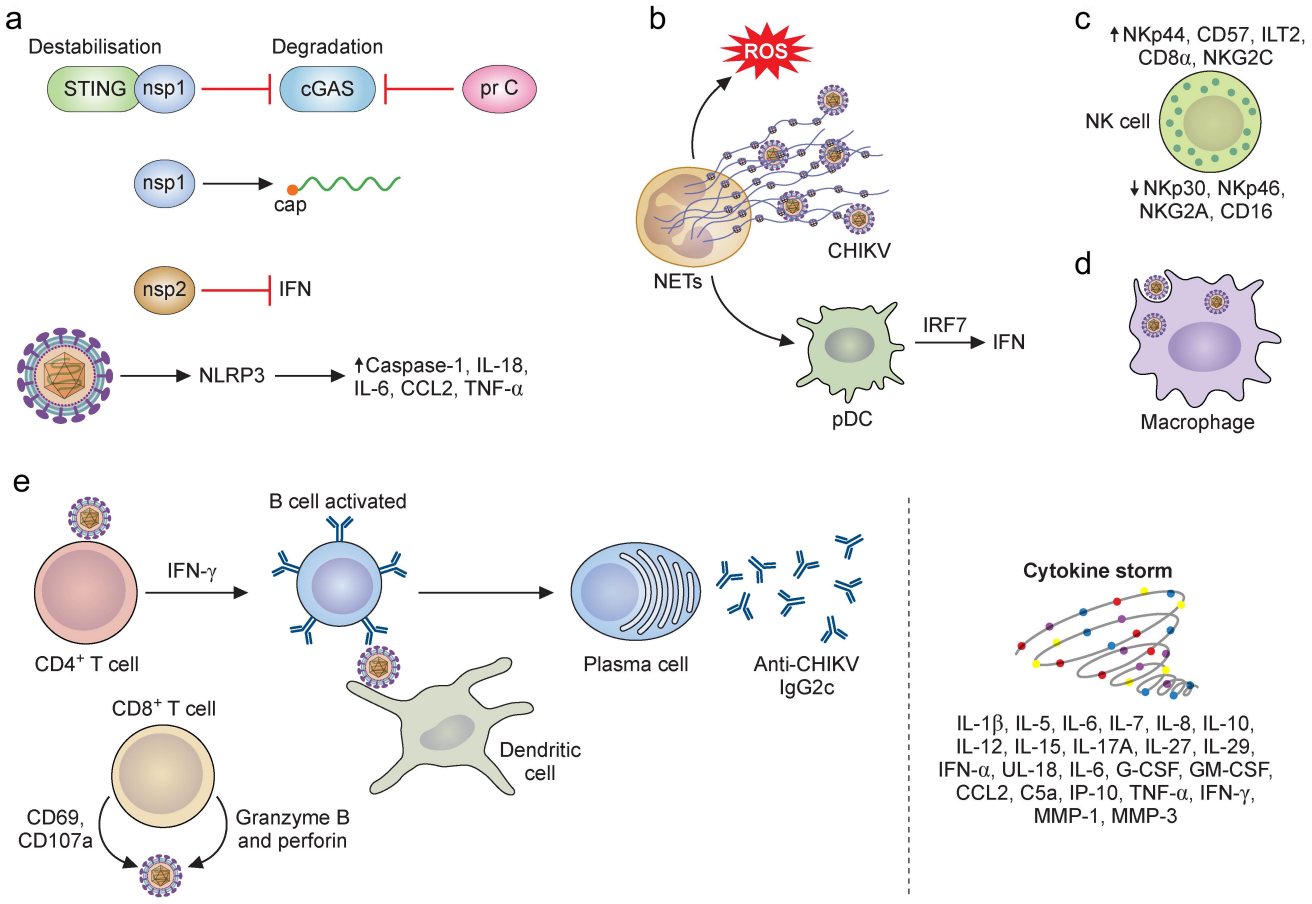

Figure 5. Immune activation and evasion by chikungunya virus.

(a) CHIKV can evade the immune system by destabilization of STING (via nsP1) and degradation of cGAS (via the capsid), by mimicking cellular capped mRNA (via nsP1), by blocking IFN activation (via nsP2), or by activating the inflammasome. (b) Neutrophil extracellular traps (NETs) activate plasmacytoid dendritic cells (pCaDCs), which recognize CHIKV and produce high levels of IFN-I in response to CHIKV infection through IRF7 activation. (c) Natural killer cells possess specific molecular surface signatures in CHIKV infection. (d) Macrophages serve as reservoirs for CHIKV. (e) Elimination of CHIKV by T $CD8^+$ cells. T $CD4^+$ cells produce IFN-Y leading to the activation of B cells and the production of specific IgG antibodies.

protein levels, and inflammatory markers, demonstrating potential for therapeutic development against CHIKV infection [219].

Activation of host PRRs initiates signalling cascades that trigger the host antiviral response, including induction of interferon signalling. Type I IFNs, including IFN-α and IFN-β, play a central role in the host's defence against CHIKV. Mice partially deficient in type-I interferon (IFN-α/βR$^{\pm}$) develop mild CHIKV-induced disease, whereas mice completely deficient in type-I interferon (IFN-α/βR$^{-/-}$) develop severe disease characterized by muscle wastage, lethargy, and rapid death compared with resistant wild-type mice [157]. In addition, IFN-γ, interleukins, and tumour necrosis factor-alpha (TNF-α) play essential roles in regulating the immune response against CHIKV infection and protecting host from disease [220–223]. In fact, infection with CHIKV in IRF3/7$^{-/-}$ KO mice resulted in a drastic decrease in the levels of IFN-γ and TNF-α, leading to the death of mice [158].

Interferon-stimulated genes (ISGs) have also been shown to play a role in CHIKV infection and disease with ISG15 identified as a crucial component regulating CHIKV disease in neonatal mice. In the absence of ISG15, mice were highly susceptible to CHIKV infection, showing increased proinflammatory response with no changes to viral load compared with WT mice, highlighting a role as an immunomodulatory molecule in regulating the innate immune response [224].

The inflammasome has also been found to play a role in CHIKV infection, with NLRP3 inflammasome activated in both CHIKV-infected humans and mice, leading to inflammation with high levels of caspase-1 and interleukin-18 gene expression (Figure 5) [225]. Inhibiting NLRP3 with MCC950 in a mouse model reduced IL-6, CCL2, and TNF-α levels leading to the decrease of CHIKV-induced inflammation, bone loss, and myositis without affecting viral replication [225]. Moreover, CHIKV increases IL-1β expression and promotes caspase-1 maturation, confirming its capacity to trigger inflammasome [156]. Most notably, silencing caspase-1 was observed to enhance CHIKV replication in dermal fibroblasts, emphasizing the skin's role in creating a pro-inflammatory and antiviral environment by initiating the inflammasome early in response to CHIKV infection [156].

4.2. Soluble factors and immunopathogenesis

One of the primary outcomes of PRR activation and interferon stimulation is the induction of soluble factors such as cytokines, chemokines, and complement, all of which contribute to CHIKV immune response and disease pathogenesis [226–228]. The balance between pro- and anti-inflammatory factors varies between individuals and is known to affect disease severity in viral infection in general [229].

CHIKV-infected patients express high levels of pro-inflammatory factors (Figure 5) such as interleukins (IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18), interferons (IFN-α and IFN-γ) [230–232], granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α [233,234], and chemokines (CCL2, CCL3, and CXCL10) [235,236]. These pro-inflammatory factors are instrumental in recruiting immune cells to the site of infection, initiating inflammation, and facilitating the adaptive immune response against CHIKV [222,230,237].

Studies focusing on soluble factors in patient cohorts have helped to uncover CHIKV disease-state biomarkers. Patients with varying symptom severity exhibit differences in immune markers, with severe disease associated with higher circulating levels of MCP-1/CCL2 and IL-6 and lower levels of IL-8 compared to mild cases [238]. One study focused on the very early stages of infection found increased levels of key immune mediators like MIG, CXCL10/IP-10, and Complement C5a anaphylatoxin [239]. Interestingly, low levels of TNFα, IL-13, IL-2, and IL-4 during the acute phase were found to be predictive for the development of chronic joint pain, indicating the importance of a robust cytokine response for viral clearance and protection against chronic arthritis [240]. Studies focused on chronic disease have found the persistence of arthralgia in CHIKV patients to be associated with elevated levels of IL-1β, IL-6, IL-8, MCP-1/CCL2, MMP-1, MMP-3 [241], and GM-CSF [242]. Another study identified elevated levels of IL-17A, IL-27, and IL-29 in chronic CHIKV disease patients, with IL-27 particularly linked to chronic sequelae, supporting the theory that an inflammatory pathology underlies the persistent joint-symptoms experienced by some CHIKV-patients [243].

The association of soluble factors and pain severity has also been studied in patient cohorts, with severe pain being linked to higher TNF-α, IL-12, and MCP-1/CCL2 levels [233], while trends in elevated IFN-γ, IL-1β, IL-6, and IL-8 further highlight their association with disease severity [241]. Altogether, these findings underscore the dynamic pro-inflammatory immune response and complement system activation in the immunopathogenesis of CHIKV infection.

4.3. The function of innate immune cells in CHIKV disease

During the acute phase, dendritic cells, macrophages, neutrophils, natural killer (NK) cells, and other lymphocytes infiltrate the primary sites of infection, particularly the joints and muscles, leading to the swelling of synovial cells and surrounding vessels, causing arthralgia in patients [161,244]. This cellular influx is mediated by a rich milieu of pro-inflammatory cytokines and chemokines at the infection site, including CCL2/MCP-1 [234,245], which are known to play a key role in the pathogenesis of CHIKV-induced disease [233]. CCL2/MCP-1 is produced by a range of cell types, predominantly macrophages. Studies have demonstrated that macrophages can also potentially act as reservoirs for CHIKV, facilitating CHIKV prolonged persistence during the course of the infection (Figure 5) [161,246]. CHIKV also infects blood monocytes, leading to a peak in viraemia during the acute phase of infection [247].

There have been limited studies focusing on the role of dendritic cells (DCs) in CHIKV infection. In a primate model of CHIKVD, DCs were found to be susceptible to infection [161], however infection of human primary DCs *in vitro* has proven to be challenging [89]. In terms of driving infection, intracerebral infection of neonatal mice has shown that astrocytes are highly susceptible to infection and CD206+ DCs influx occurs early at the site of infection likely triggering the innate immune response [248]. Plasmacytoid DCs (pDCs) were found to sense CHIKV-infected cells indirectly leading to the activation of IRF7 and the production of IFN-I [249], which was effective in protecting against lethal CHIKV infection in a mouse model. This emphasizes the crucial role of pDC IRF7 signalling in guiding both Type I and Type II IFN responses during CHIKV infections.

Neutrophils appear to be key mediators of arthritic symptoms and tissue damage, releasing antimicrobial molecules and reactive oxygen species (ROS) (Figure 5) in CHIKVD [250]. They can also form neutrophil extracellular traps (NETs) through a process called NETosis, which have an anti-CHIKV effect [251]. NETs activate pDCs, which, as mentioned above, recognize CHIKV and produce high levels of IFN-I in response to CHIKV infection through IRF7 activation [249,252,253].

NK cells play a critical role in CHIKV defence, with notable surface receptor changes during infection (Figure 5). This involves increased NKp44, CD57, ILT2, CD8α, NKG2C expression and decreased NKp30, NKp46, NKG2A, CD16 [254]. Rapid NKG2C expansion in the acute phase results in strong cytolytic activity [254]. In a separate study on NK and NKT-like cells, chronic CHIKV patients displayed reduced NKT-like cell percentages and lowered NK cell markers (NKp30+, CD244+, DNAM-1+, and NKG2D), with increased CD94+ and NKG2A in NKT-like cells compared to recovered individuals [255]. Chronic patients had elevated IFN-γ and TNF-α in NKT-like cells, while recovered individuals exhibited increased IFN-γ in NK cells [255].

4.4. Adaptive immune responses

Adaptive immunity is classically separated into two arms: the cellular (cells; predominantly T and B cells) and humoral (antibodies), both of which play a critical role in the immune response to CHIKV infection (Figure 5). The cellular immune response to CHIKV is a complex involving the activation of various immune cells, of which CD4+ T cells are perhaps the most well studied [256]. In terms of CHIKVD, studies have shown CD4+ T cells are key drivers of CHIKV-induced inflammation. In mouse model studies, CHIKV-infected $CD4^{-/-}$ mice develop less inflammation than WT or $CD8^{-/-}$ mice and this CD4+ driven inflammation was found to be IFN-γ-independent [257]. A further study found that T cell receptor-deficient CHIKV-infected mice develop joint inflammation only after the addition of splenic CD4+ T cells from CHIKV-infected wild-type mice [258]. This study and others highlight the impact of CD4+ T cells on joint damage associated with CHIKV infection [177,258,259]. Despite CD4+ T cells being identified as key cellular drivers of joint damage during CHIKV infection, they are necessary for generating memory CD4+ T cells [165,260], which is critical for anti-viral adaptive immunity. Additionally, $CD4^{+}$ T cells are responsible for IFN-γ expression in response to CHIKV infection leading to the stimulation of B cells and the production of CHIKV-specific antibodies IgG2c [260], promote the activation and proliferation of other immune cells, and enhancing the cytotoxic activity of CD8+ T cells [261].

The role of CD8+ T cells in eliminating virus-infected cells during CHIKV infection remains unclear. During acute CHIKV infection in mice, CD8+ T cells infiltrate the joint space; however, their function appears to offer no protection against joint damage with CD8+ deficient mice developing similar CHIKV-induced joint pathology [257]. In acute human CHIKV-infection, CD8+ T cells have been found to express markers associated with T cell activation,

including CD69, CD107a, granzyme B, and perforin [230,262,263], suggesting a role in adaptive immune activation and viral clearance, however this is yet to be conclusively proven. Persistent CHIKV infection has been observed in mouse footpads for up to 16 weeks post-challenge [264]. Such persistent activation can eventually lead to CD8+ T cell exhaustion, characterized by decreased levels of CD8+ T cells and reduced expression of the activation marker CD69, as seen in chronic CHIKV patients [262]. In support of this, Davenport et al., recently demonstrated that CHIKV induces and sustains a long-lasting infection within tissues associated with joints by evading the immune response of CD8+ T cells [265]. Overall, the role of T cells in CHIKV infection is complex and has been extensively reviewed by Poh and colleagues [266].

During the early stages of CHIKV infection, B cells produce IgM antibodies in response to the virus [267]. IgM antibodies exhibit a preference for binding to and targeting epitopes found on the surface glycoproteins E1 and E2 of CHIKV. The presence of neutralizing IgM antibodies at day 6 is associated with lower viraemia levels [267]. Beyond this stage, the overall neutralizing capacity is mainly attributed to the IgG antibody response. A recent study has shown that CHIKV infection provides cross-neutralizing antibodies and memory B-cells against other arthritogenic alphaviruses and that memory B-cells against CHIKV can last up to 24 years post infection [268].

The interaction between CHIKV antigens and B cell receptors (BCRs) triggers B cell activation [269], involving various signals, including cytokines and co-stimulatory molecules, leading to B cell proliferation and differentiation (Figure 5). Activated B cells differentiate into plasma cells, which then produce antibodies specifically targeting CHIKV [268,270], primarily directed against the viral envelope proteins [260,268,271]. These antibodies have been shown to recognize and bind to specific epitopes in E2 such as three conserved peptides, P3 and P4 (located in Domain A) and P5 (located in Domain B) [271]. The CHIKV E2 glycoprotein has been recognized as the most immunodominant protein of the CHIKV virion, with CHIKV-neutralizing IgG antibodies binding to the E2 glycoprotein, preventing its attachment and entry into host cells [272–275], as well as blocking egress [276].

4.5. Mechanisms of target tissue damage

Based predominantly on mouse studies, CHIKV infection is known to result in a range of organ and tissue damage (as reviewed by Traverse et al.) [277]. As discussed in previous sections, the hallmark clinical features of CHIKV are arthritis, arthralgia, myalgia, fever, and occasionally rash. Gastrointestinal upset, cardiac symptoms, respiratory issues, and neurological complications have also been reported but are considered atypical [278–282]. Following a primary viraemia, CHIKV reaches target organs including but not limited to the skin, muscles, bones, and joints [164,283]. Although the exact mechanisms that result in the tissue damage characteristic of CHIKVD are not fully understood, it is known that CHIKV infection drives a powerful inflammatory process resulting in high levels of pro-inflammatory cytokines. These include IL-1β and TNF-α which are believed to drive tissue damage, particularly in the joints [222,223,284].

In the tissues of the joints (muscle, bone, cartilage, and tendons), the virus is known to replicate to high titres directly infecting synovial fibroblasts, chondrocytes, osteoclasts/osteoblasts, and myocytes leading to inflammation and damage [228,285,286]. CHIKV infection has been shown to alter the RANKL/OPG ratio, resulting in bone loss [286]. Similarly, infection results in a loss of proteoglycans in the cartilage matrix, resulting in cartilage degradation [287]. CHIKV infection of the muscle has been shown to be critical for the development of disease symptoms [167], and cells of the muscle (myoblasts, fibroblasts) might act as a source of persistent viral RNA, therefore contributing to chronic disease symptoms [155]. Furthermore, infection of myoblasts of the skeletal muscle upregulates inflammatory cytokines such as MCP-1, IP-10, MIP-1α, and IL-8 [288], which likely contributes to muscle pain (myalgia) and weakness [167].

CHIKV-induced neurological disease includes meningitis, encephalitis, and Guillain Barre [289] and has proven difficult to study. A recent study examining CHIKV strain differences in immunocompetent mice found that similar to arthritis disease, neurological disease is driven by CD4+ T-cell infiltration and proinflammatory cytokine response [290].

There is growing evidence that CHIKV infection results in cardiovascular complications, including myositis and cardiac arrhythmias [281]. Using primary human cardiac cells and small animal models, a recent study has shown that CHIKV targets cardiac fibroblasts, and that mitochondrial antiviral-signalling protein (MAVS) signalling enables viral clearance and protects from cardiac damage [228].

5. Virulence determinants at the virus-host interface

Viral virulence is a complex trait influenced by multiple factors, including viral proteins, viral genetic diversity, and mutations. This section will explore the different

determinants of CHIKV virulence at both the virus-vector and the virus-host interface.

5.1. Viral genetic diversity and mutations affecting transmission

CHIKV is genetically diverse, consisting of four major lineages: the Asian Urban (AsU) lineage, the Eastern-Central-South-African (ECSA) lineage, the West African (WA) lineage, and the Indian Ocean lineage (IOL) [2,291,292]. These lineages, which differ in pathogenesis [165], can be defined not only by nucleotide and amino acid differences but also by the length and composition of the 3'UTR [58,293]. Specifically, the WA lineage contains the shortest 3'UTR, while the Asian Lineage and viruses found during the 2013 Caribbean outbreak contain the longest 3'UTR [294–296]. These differences in 3'UTR are important for replication and adaptation in mosquitoes [294,295]. In addition to the untranslated regions, CHIKV has undergone extensive evolution throughout the last several decades leading to the emergence of CHIKV strains that have contributed to increased transmission and virulence. Perhaps the most famous adaptive mutation was a single-point mutation in the E1 glycoprotein (A226V) that emerged in the 2005–2006 outbreak on the island of La Réunion in the Indian Ocean [297] (Figure 1f). This single-point mutation allowed for increased transmission of CHIKV by the *Ae. albopictus* mosquito [298]. Since this outbreak, CHIKV has continued to evolve both in nature and in laboratory studies [81,299], and new emerging variants have been identified that contribute to viral fitness and virulence [81,299,300]. Notably, the mutation E2 L210Q further enhanced viral fitness in *Ae. albopictus* mosquitoes [301]. Despite *Ae. albopictus* prevalence in Southeast Asia, the E1 A226V variant was absent on the Asian lineage background due to an epistatic interaction between E1 residues 226A and 98T [301]. Additionally, novel mutations, E1 K211E and E2 V264A, circulating in India and France, enhanced infectivity in *Ae. aegypti* mosquitoes [302,303]. Taken together with climate change in mind, these factors coupled with the increased spread of these vectors are poised to sustain CHIKV as a significant global public health threat for years to come.

5.2. Viral determinants of CHIKV antagonism and evasion of the immune system

CHIKV has evolved mechanisms to both antagonize host antiviral immunity and evade host immune responses. Over the past few decades, studies have been dissecting the roles of CHIKV viral proteins and the mechanisms used to promote infection and escape viral clearance.

CHIKV nsP1 contributes to both antagonism of antiviral response and evasion of the host defence mechanisms through several strategies (Figure 5). The 5'-end capping activity of nsP1 modifies viral RNAs to mimic cellular mRNA [105,304]. nsP1 also modulates host cell processes to favour viral replication, such as regulating cholesterol homoeostasis [108]. Maintaining cholesterol balance is critical for facilitating CHIKV genome replication. Additionally, CHIKV nsP1 has recently been demonstrated to be a viral determinant of CHIKV virulence by regulating the IFN-I response [305]. nsP1 was found to interact with the cytosolic RNA sensor protein "Stimulator of Interferon Genes" (STING), leading to its stabilization and the degradation of the protein cyclic GMP-AMP Synthase (cGAS) (Figure 5). These processes result in the inhibition of the IFN-I response pathway and an increase in palmitoylated nsP1, a crucial post-translational modification in CHIKV replication and pathogenesis [306,307].

Both CHIKV nsP1 and nsP2 can also disrupt innate immune signalling pathways, thereby inhibiting the expression of antiviral genes (Figure 5). This includes nsP2 interference with the nuclear factor-kappa B (NF-κB) induction pathway by MDA5/RIG-I, which regulates pro-inflammatory cytokines [308]. Interestingly, a recent study has revealed that CHIKV induces the degradation of cGAS via the capsid protein C [305]. Furthermore, mutations in nsP1 (R532H) and nsP2 (E515V) were found to increase IFNα/β and pro-inflammatory cytokines *in vivo*, highlighting the role of nsP1/2 in impairing the production of type I interferons (IFN-α and IFN-β) [309]. The mutation from proline to serine at position 718 (P718S) in CHIKV nsP2 inhibits the JAK-STAT pathway by blocking STAT1 phosphorylation and its nuclear translocation, resulting in decreased IFN-induced JAK-STAT signalling in African green monkey kidney (Vero) cells [310]. Already well established in the Alphavirus genus, CHIKV nsP2 induces host cell shutoff, a process that inhibits host gene expression [311,312]. This can assist the virus in evading host immune responses by preventing the production of antiviral factors. Mutations in the VLoop (^{674}ATLG^{677}) of CHIKV nsP2 reduce its transcriptional activity and interfere with CHIKV subversion of the activation of the cellular antiviral response [313].

Similarly, nsP3 is multifunctional, possessing an ADP-hydrolase activity and playing roles in replication complex formation, host cell shutoff, and immune

evasion [314]. Interestingly, in most CHIKV strains, the C terminus of the viral nsP3 gene contains a conserved opal stop codon [315]. The Opal524R mutation in CHIKV nsP3 does not impact viral replication kinetics but leads to significantly reduced swelling, inflammation, and damage in the feet of infected mice. It also delays the onset of proinflammatory cytokines and chemokines and reduces the recruitment of CD4+ T cells and Natural Killer cells [61].

Finally, TF acts as an interferon antagonist, diminishing early IFN-1 expression [141], which is likely why TF is a critical factor for virulence [120,316–318]. However, the mechanisms by which TF interferes with innate immunity are still unknown.

In addition to the role of CHIKV proteins in the antagonism of host immune response, CHIKV has several mechanisms of immune evasion. It is hypothesized that intercellular long extension (ILE) formation and cell-to-cell transmission helps CHIKV evades neutralizing antibodies (Nabs) [142,150]. While nAbs can block viral egress and disrupt cell-surface spike proteins [64,319], they are less effective against cell-to-cell transmission, allowing nearby cells to become infected even in the presence of high nAbs concentrations [64,150,320]. Furthermore, ILE-mediated cell-to-cell transmission was demonstrated to occur *in vivo* through adoptive transfer of infected cells in the presence of nAbs [146]. In addition, CHIKV evades the immune response in fibroblast cells by inducing translational shutdown and causing cell apoptosis [321]. Evidence suggests that CHIKV is transmitted through apoptotic bodies [93] and exosomes [322], highlighting multiple egress mechanisms that would enhance infection and dissemination to cell-to-cell.

CHIKV E2 segments have been implicated in viral persistence, exemplified by the E2 K200R mutation enhancing viral evasion of phagocytic clearance by scavenger receptor MARCO, leading to increased viraemia and dissemination [323]. In infant mice, the E1 variant V80I:A129V caused heightened viral loads and 100% lethality after 6 days of infection [300]. Mechanistically, these mutations boost fusion and particle stability *in vitro*. It is important to note that the interaction between these viral determinants of virulence and host factors, such as host genetics and immune responses, plays a critical role in the overall clinical outcome of CHIKV infection [324].

5.3. Entry factors that influence infection and disease

It is likely that CHIKV and other GAG-binding viruses evolved to maintain a low to moderate affinity for cell-surface GAGs to enhance interactions with the cell surface during entry but still allow for efficient egress from cells [325] and possibly immune evasion [326]. The biological significance of binding to GAG attachment factors *in vivo* has not been fully established. A CHIKV vaccine strain (181/25) or clinical strains encoding mutations in the E2 attachment protein (e.g. E79K, G82R) that enhance binding to GAGs [69,70,327,328] are attenuated in mouse models [327–330]. Attenuation occurs through multiple mechanisms, including eliciting dampened immune responses and tissue pathology [328,331–333]. Ander and colleagues recently observed that GAGs present on phagocytic cells mediate interactions with 181/25 and are required for the HS-dependent clearance of the virus from blood, supporting the notion that affinity of CHIKV-GAG interactions modulate virulence [326]. Thus, identifying and characterizing the viral residues that mediate GAG binding is essential to study CHIKV-GAG interactions.

Engagement of CHIKV with entry receptor MXRA8 is important for CHIKV pathogenesis in a mouse model of the disease. Preincubation of CHIKV with an Fc-tagged MXRA8 decoy reduced viral titres and swelling [78]. Likewise, pretreatment of mice with an anti-Mxra8 blocking antibody reduced viral titres and inflammation at the site of injection [78]. *Mxra8* KO-infected mice also displayed altered immune cell infiltration and reduced proinflammatory mediators [84], suggesting either a direct engagement of MXRA8 and subsequent signalling or infection of cells that normally express MXRA8 (e.g. muscle cells) [167] moderates immune responses to CHIKV infection. In addition, infection of WT mice with a virus harbouring a mutation (E2 D71A) that abrogates binding to MXRA8 resulted in a modest reduction (<15-fold) reduction in viral titres in joint-associated tissue [84]. Together, these data suggest that expression of MXRA8 is required for maximal viral replication, dissemination, host immune responses, and virulence [84]. However, since MXRA8 is not expressed on all cell types targeted by CHIKV (e.g. endothelial cells, hepatocytes, keratinocytes, and macrophages) [86], and CHIKV replication in mice was attenuated but not abolished when MXRA8-CHIKV interactions were disrupted, CHIKV likely engages additional putatively identified or as yet undiscovered entry receptors.

Other possible entry receptors include members of the C-type lectin receptors (CLRs), a large family of pattern recognition receptors (PRRs) expressed predominantly on the PM of immune cells, including monocytes, macrophages, and dendritic cells (DCs), which

can bind glycans present on viral pathogens [334]. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), liver/lymph node-specific intracellular adhesion molecules-3 grabbing non-integrin (L-SIGN), and mannose receptor (MR) have been implicated in CHIKV replication or pathogenesis [67,160,335–338]. Exogenous expression of DC-SIGN and L-SIGN in mammalian cells enhances infection of CHIKV [67] or CHIKV-pseudotyped viruses [335]. Many of these CLRs recognize high-mannose glycans, which can be found on CHIKV virions isolated from mosquito or insect cells [334,339,340]. Direct binding between soluble monomeric CHIKV E2 isolated from insect cells and both DC-SIGN and MR has been observed [160], suggesting these CLRs can recognize and bind high-mannose displayed on mosquito-derived CHIKV. It is possible that CHIKV deposited in the skin during a blood meal could specifically bind to CLRs on dermal cells, which could in turn influence tropism and early immune responses during infection in mammalian hosts. In line with this, $CD14^{+}$ dermal antigen-presenting cells isolated from the human dermis are permissive to CHIKV, and infection can be blocked by pretreatment of cells with mannan, an inhibitor of CLR-mannose binding [160]. However, mechanisms of CLR-mediated entry and induction of immune signalling have not been thoroughly investigated for any alphavirus.

5.4. Host genetic factors influencing susceptibility to CHIKV

CHIKV susceptibility and the severity of the infection can vary among individuals depending on host genetic factors. To date, only a few genetic markers have been linked to differences in CHIKV infection or disease severity. Some human leukocyte antigen (HLA) alleles have been associated with increased susceptibility or resistance to CHIKV. For example, the presence of the HLA-DRB1 × 04-HLA-DQB1 × 03 haplotype increases susceptibility to CHIKV infection, while the presence of the HLA-DRB1 × 11 and HLA-DRB1 × 11-HLA-DQB1 × 03 haplotypes favours resistance to CHIKV infection [341,342]. Chaaithanya and colleagues have shown a lower frequency of HLA-DQB1 × 03:03 in CHIKV-infected patients compared to healthy patients [341].

The use of computational tools like NetMHCII to predict the binding affinities of CHIKV CD4 epitopes has uncovered that HLA-DQ molecules exhibit a greater capacity to bind a larger number of CHIKV peptides compared to HLA-DRB1 molecules. These findings raise the possibility that HLA-DQB1 alleles, along with critical variations in amino acid composition within the peptide-binding pockets, may exert a notable influence on susceptibility to CHIKV infection and its pathogenesis.

Other than the HLA complex, genetic studies in CHIKV infection have also focused on other genes such as the 2'-5'-oligoadenylate synthetase (OAS) gene cluster and CD209 (DC-SIGN). OAS activation by IFNs inhibits viral replication [343,344], while CD209 in macrophages and dendritic cells triggers innate immunity [345]. In India, the CD209 gene variant rs4804803 was found to be related to CHIKV susceptibility [341] and the OAS gene cluster variants (OAS1 rs1131454, OAS2 rs1732778, rs15895, and OAS3 rs2285932), influenced CHIKV-related symptom development. CD209 variants affect transcription factor binding and DC-SIGN expression, impacting dendritic cell responses and CHIKV susceptibility [338]. Interestingly, the G-G CD209 variant is linked to higher CHIKV susceptibility, while G-A variants may offer reduced likelihood of developing fever as a result of decreasing the inflammatory response.

Single nucleotide polymorphisms (SNPs) in TLR-3, TLR-7, and TLR-8 can impact receptor–ligand interactions, processing, and expression, and influence immune responses against CHIKV infection. Three SNPs of TLR-7 (rs179010, rs5741880, and rs3853839) have been found to be related to CHIKV-induced symptoms such as fever, joint pain, and rash [346]. The SNP in TLR-3, rs3775290, was associated with joint pain, while the SNP rs3764880 in the TLR-8 gene was associated with fever. Sengupta and colleagues compared the role of TLR-3, −7, and −8 SNPs in mono- and co-infected Indian patients with Dengue virus (DENV) and CHIKV [347], and uncovered distinct patterns in the distribution of SNPs in these genes. Individuals with CC genotypes of TLR-7 and TLR-8 SNPs were notably more susceptible to co-infections, while genotypes CT genotype of TLR-8–rs5744080 and AG genotype of TLR-8-rs3764880 provided a level of protection against co-infections. Interestingly, co-infected patients with the TT-rs179010 genotype exhibited high DENV viral load, whereas other genotypes (AA genotype of TLR7-rs179008 and GG genotypes of TLR8-rs3764879 and rs3764880) were associated with elevated CHIKV viral load. This study underscores the significance of TLR-7 and TLR-8 SNPs in determining susceptibility to both mono- and co-infections of DENV and CHIKV, with specific genotypes playing a role in influencing the viral load in co-infected patients. Genetic susceptibility to CHIKV infection is likely influenced by a combination of multiple genetic

factors rather than a single gene. Additionally, genetic factors may be influenced by environmental factors, such as viral strain, mosquito vector, and host immune status, to dictate susceptibility and disease severity. The role of host factors has been reviewed by Rueda and colleagues [324].

5.5. Genetic screening to identify host factors as therapeutic targets

Host factors play essential roles in various aspects of CHIKV replication. Genetic screens help identify critical host proteins that have potential for the development of host-targeted therapeutics. A few are highlighted here. In a 2015 study, the cellular response to CHIKV infection was investigated in derived-nonhuman primate cells 293/ACE2 cells using advanced stable isotope labelling by amino acids in cell culture (SILAC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques [348]. This study identified 106 downregulated host proteins at 12 h post-infection, including decreases in key proteins such as Rho family GTPase 3 (Rnd3), DEAD box helicase 56 (DDX56), polo-like kinase 1 (Plk1), and ubiquitin-conjugating enzyme E2C (UbcH10). Interestingly, when these cellular factors were overexpressed, the susceptibility of cells to CHIKV-infection was reduced, suggesting that these proteins benefit CHIKV replication [348].

In a separate study, a genome-wide siRNA screen was conducted to elucidate the intricate interplay between CHIKV, and host factors identified 156 proviral and 41 antiviral host factors [349]. Subsequent analysis into the cellular pathways associated with identified human proviral genes found several druggable targets, with 21 inhibitors targeting six distinct proviral factors or pathways demonstrating robust antiviral activity against CHIKV while exhibiting low toxicity to host cells. Notably, the combination of two inhibitors, the calmodulin inhibitor pimozide and the fatty acid synthesis inhibitor TOFA, exhibited antiviral effects in mouse models [349]. Furthermore, a virus overlay protein binding assay (VOPBA) and matrix-assisted laser desorption/ionization time-of-flight analysis (MALDI TOF/TOF) identified CHIKV binding proteins in HEK-293T and Vero-E6 cells [350]. STAT-2 was observed to bind CHIKV in Vero-E6 cells, while HSP70 and actin bound CHIKV in both HEK-293T and Vero-E6 cell lines, demonstrating host factors can be cell-specific.

Finally, a recent study has found that the four-and-a-half LIM domain protein 1 (FHL1) is a key host factor involved in CHIKV permissiveness and pathogenesis in humans and mice [351]. Notably, this host factor specifically increases CHIKV and ONNV infection, as its deletion decreases only CHIKV and ONNV infectivity but not other alphaviruses. The mechanism of FHL1 is through direct interaction with the hypervariable domain of nsP3, and enhancement of vRNA replication. Importantly, $Fhl1^{-/-}$ KO mice infected subdermally with 10^5 PFU of CHIKV displayed no detectable levels of infectious CHIKV particles at day 7 post infection nor any CHIKV-induced muscle pathology compared to CHIKV-infected WT mice, suggesting an essential role of FHL1 in CHIKV infection [351]. Building on this, a deeper understanding of the intricate interactions between viral components and host proteins can potentially lead to the development of more significant effective antiviral treatments for CHIKV infection.

6. Combatting CHIKV: Therapeutics, vaccines, and vector control

6.1. Current treatment strategies

As there are no specific antiviral medications approved for the treatment of CHIKV infection, current treatment strategies focus on managing symptoms and providing supportive care, including pain relief, anti-inflammatory medications, hydration, and psychological support [352,353] (Figure 4). The cornerstone of CHIKVD treatment is alleviating the often severe and debilitating symptoms experienced by patients. The use of simple analgesics such as non-steroidal anti-inflammatory drugs (NSAID; e.g. ibuprofen) and paracetamol (acetaminophen) is commonly recommended to reduce fever and relieve joint pain and muscle pain [354]. Aspirin is not recommended in the acute phase as the clinical presentation is similar to that of DENV infection, and aspirin is well known to increase bleeding and could worsen dengue haemorrhagic fever [355]. In cases of more severe disease, the use of disease-modifying antirheumatic drugs (DMARDs) has been trialled, including hydroxychloroquine and methotrexate [356], which are immunosuppressive and immunomodulatory agents that slow down disease progression by interfering with essential pathways in the inflammatory cascade. The use of these medications is cautioned in acute disease as they may exacerbate symptoms.

6.2. Development of CHIKV antiviral therapies

Antiviral therapies and vaccines are essential components of the strategy to combat CHIKV infection, and

several potential antiviral therapies are under investigation [357], as extensively reviewed by Battisti and colleagues [358] and Hucke and Bugert [357]. Very recently, 3-methyltoxoflavin has been identified as an inhibitor of CHIKV and other viruses [359] as well as Itraconazole [360]. These two potent inhibitors of CHIKV replication are promising and could be repurposed as broad-spectrum antivirals. Advances in our understanding of molecular mechanisms of viral replication and virulence are essential for the development of efficacious CHIKV-specific antivirals.

Monoclonal antibodies (mAbs) targeting CHIKV have been developed and tested in mice to evaluate safety, efficacy, dosing, and mechanisms of action before advancing to human clinical trials. For instance, human mAb DC.271B targeting the CHIKV E2 epitope conferred high protection, while other mAbs targeting CHIKV E1 were inefficient [361]. Another mAb, 19–1, which is an IgG2b κ-chain isotype targeting CHIKV E2, has a strong binding affinity and high sensitivity against CHIKV [362]. A very interesting mAb strategy developed by Moderna is the lipid nanoparticle-encapsulated messenger RNA-1944 (mRNA-1944) encoding the heavy and light chains of a CHIKV-specific monoclonal neutralizing antibody [363]. The randomized phase I took place from January 2019 to June 2020 to evaluate its safety and pharmacological activity. Although very positive results were collected in phase I, Moderna has decided not to further study this candidate in phase II clinical trial [364].

6.3. CHIKV Vaccine development

Developing a reliable and efficacious CHIKV vaccine is crucial for global CHIKV control as a robust humoral response is associated with clearance and protection from infection [260]. While there are significant challenges to the development of a CHIKV vaccine, including CHIKV disproportionality affecting predominantly poor countries and being notorious for unpredictable outbreaks, having a vaccine ready for deployment during outbreaks is vital, especially in urban communities.

Several CHIKV vaccine candidates have been developed and have progressed through various stages of preclinical and clinical testing [365] including phase III clinical trials. Inactivated or live-attenuated CHIKV vaccines are among the early vaccine candidates, and several of them have already been well reviewed [366–371]. As of October 2023, two promising vaccine candidates, VLA1553 (Valneva) and PXVX0317 (Bavarian Nordic A/S (OMX: BAVA)), have successfully passed phase III clinical trials.

Vaccine VLA1553 is a live-attenuated vaccine that has been in the development for a number of years showing a high tolerance in low- and medium-dose groups, which comes with a high level of immunogenicity and seroconversion [372]. This vaccine recently reached its primary and secondary endpoints in a phase III double-blind, placebo-controlled clinical trial and is now FDA approved and commercially available in the USA [373]. In the phase III clinical trial (ClinicalTrials.gov ID: NCT04546724), involving 4128 participants, the VLA1553 vaccine candidate showed safety and immunogenicity up to day 180 after vaccination with a high level of neutralizing antibodies (98.9% of participants) peaking at 28 days post-vaccination and remaining steady for the 180-day duration of the study. However, common side effects mirrored CHIKV symptoms including fatigue, headache, and muscle and joint pain, with severe CHIKV-like adverse reactions in 1.6% of vaccine recipients. VLA1553 was approved for use in the US by the Food and Drug Administration in late 2023 for adults at increased risk of exposure to CHIKV, with a warning that the vaccine may cause prolonged CHIKV-like adverse reactions [374]. Currently, VLA1553, under the licenced name IXCHIQ® [375] is recommended by the CDC for adults travelling to regions with known CHIKV outbreaks. At the time of writing, the CDC is recommending vaccination for those travelling to Timor-Leste, where there is increased CHIKV activity [376].

Vaccine PXVX0317 is a virus-like particle (VLP) vaccine that has been studied for the last few years. Already studied in a phase II clinical trial, PXVX0317 conferred high immunization against CHIKV through neutralizing antibodies and circulating antigen-specific B cells in 20 participants [377]. More importantly, the vaccine stimulated the production of broadly neutralizing mAbs that have a unique binding affinity to the E2 B domain and display potent neutralization of other related arthritogenic alphaviruses. Additionally, PXVX0317 has recently finished its randomized, multicentre, double-blind, and placebo-controlled phase III clinical trial which included young people aged from 12 to 64 (ClinicalTrials.gov ID: NCT05072080). In this study involving 3254 healthy participants, a single intramuscular injection of PXVX0317 or a placebo was administered [378]. The results, observed within 22 days after vaccination, indicated that PXVX0317 was highly effective at inducing CHIKV-neutralizing antibodies in 98% of those who received the active vaccine. The primary objective of the study was to determine the level of seroprotection and the neutralizing antibody levels that met or exceeded the established threshold for seroprotection. PXVX0317 also generated

neutralizing antibodies in 97% of participants just two weeks after vaccination, demonstrating a rapid establishment of protective immunity. Even six months after vaccination, 86% of individuals still maintained seroprotective levels of neutralizing antibodies, emphasizing the durability and well-tolerated nature of the vaccine. In addition, adverse events associated with PXVX0317 were primarily of mild or moderate severity, and the vaccine was well tolerated in both adult and adolescent populations.

The mRNA vaccine technology has emerged during the last few decades and recently demonstrated its high efficiency and safety during the COVID-19 pandemic [379–381]. In CHIKV vaccine development, a randomized placebo-controlled phase I clinical trial with a dose-ranging study was conducted in the US from July 2017 to March 2019 [382]. Healthy adults (ages 18–49) received two intramuscular injections of mRNA-1388 (25 µg, 50 µg, or 100 µg) or placebo, 28 days apart. The study evaluated the safety, tolerability, and immunogenicity of mRNA-1388 [382]. mRNA-1388 was found to be safe and well tolerated, inducing strong and persistent humoral responses with dose-dependent increases in neutralizing antibody titres, remaining elevated up to a year post-vaccination compared to placebo.

Recently, two studies conducted in mice found that mRNA-LNP vaccines elicited strong immune responses in C57BL/6 mice. One vaccine, developed in 2022, expressed the CHIKV E2-E1 antigen [383] while the other, developed in 2023, encoded the structural proteins (C-E3-E2-6K-E1) of the CHIKV LR2006 OPY1 strain [384]. These studies confirm the robust potential of mRNA-LNP vaccine technology for developing CHIKV vaccines.

6.4. Vector surveillance and control

Finally, to combat CHIKV and other arboviruses, efforts to monitor and combat mosquito vectors are critical for effectively reducing the transmission of these diseases and safeguarding public health. Monitoring *Aedes* mosquito populations in endemic areas could help predict and prepare for potential CHIKV outbreaks. Population-based surveys and seroprevalence studies help assess the extent of CHIKV exposure within a community and inform public health interventions [385,386]. Geographic Information Systems (GIS) technology is used to map CHIKV transmission patterns and identify high-risk areas, aiding in the allocation of resources for vector control and healthcare services [387]. Vector control strategies aim to reduce the population of disease-carrying mosquitoes, ultimately diminishing the risk of viral transmission to humans. One successful approach involves the release of Wolbachia-infected mosquitoes in various regions to suppress the local *Aedes aegypti* populations, thereby reducing the transmission of CHIKV and other arboviruses [388]. Additionally, other vector control methods include the use of insecticides, larvicides, and environmental modifications to eliminate mosquito breeding sites. Community engagement and education play a critical role in encouraging individuals to take preventive measures, such as using mosquito nets, repellents, and practicing good sanitation to reduce the risk of infection. Ongoing research and innovative approaches in vector control are crucial in the fight against CHIKV and other arboviruses, with the goal of reducing the burden of these diseases on public health.

6.5. Challenges and future directions in chikv research and control

Despite significant progress in understanding CHIKV pathogenesis, developing diagnostic tools, and exploring therapeutic and vaccine options, several challenges and future directions remain critical for effectively managing CHIKV infections and reducing their impact. CHIKV infection presents with symptoms like other mosquito-borne diseases, such as dengue and Zika viruses [191]. Therefore, it is essential to develop new, accurate, and rapid differential diagnostic tools that can distinguish CHIKV from other arboviruses. Additionally, understanding the impact of co-infections with multiple arboviruses on disease severity and clinical outcomes is an area of growing importance [389]. Importantly, the lack of specific antiviral drugs for CHIKV hinders treatment options, especially for severe cases. Significant progress has been made during the last decade; however, continuing research into antiviral drug development and repurposing of existing drugs may lead to better effective treatments [390].

Although CHIKV is a global health threat, many regions affected by CHIKV are middle to low-income countries with limitations to healthcare infrastructure or resources for surveillance and outbreak response [391,392]. Although challenging, collaboration between governments, international organizations, research institutions, and public health agencies is crucial for sharing knowledge, coordinating responses, and mitigating the global impact of CHIKV, especially in low-income countries. Moreover, we must make efforts to expand vaccine options and production, reduce costs, and establish distribution mechanisms that reach vulnerable populations, thus ensuring equitable access to CHIKV vaccines.

In the context of CHIKV distribution, effective vector control remains a cornerstone of CHIKV prevention [393]. *Aedes* mosquitoes are highly adaptable, and their resistance to insecticides poses a global challenge [394]. Hence, developing new alternative vector control methods, such as genetically modified mosquitoes and Wolbachia-based strategies, could prevent future CHIKV outbreaks [395]. Irreversibly, climate change is expanding the geographic range of *Aedes* mosquitoes, increasing the potential for CHIKV transmission to new areas [396]. Global effort and research should be added in the monitoring and predicting of the impact of climate change on vector distribution [36].

7. Conclusion

CHIKV continues to pose a significant global health threat due to its potential for large-scale outbreaks and the persistent challenges it presents. Understanding CHIKV's virology, transmission dynamics, pathogenesis, immune evasion mechanisms, and the development of diagnostic tools and vaccines are essential steps in mitigating its impact. Vector control remains essential in preventing CHIKV transmission, especially given the adaptability of *Aedes* mosquitoes to urban environments and the effects of climate change on their distribution. Developing effective antiviral therapies and durable vaccines is a pressing need, along with ensuring equitable access to these interventions. Ongoing research must address challenges such as accurate differential diagnosis, co-infections, and the long-term efficacy of vaccines. International collaboration, robust public health systems, and proactive surveillance are vital for early detection and effective responses to CHIKV outbreaks. In a rapidly changing world, concerted efforts across disciplines and borders are essential to combat CHIKV effectively and protect global health.

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Author Wesley Freppel contributed to the research, writing and editing of this review

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Data availability statement

Data sharing are not applicable – no new data are generated.

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