Chikungunya Virus: Current Perspectives on a Reemerging Virus

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ABSTRACT Chikungunya virus (CHIKV) is a mosquito-borne alphavirus in the family Togaviridae that causes outbreaks of debilitating acute and chronic arthralgia in humans. Although historically associated with localized outbreaks in Africa and Asia, recent epidemics in the Indian Ocean region and the Americas have led to the recognition that CHIKV is capable of moving into previously unaffected areas and causing significant levels of human suffering. The severity of CHIKV rheumatic disease, which can severely impact life quality of infected individuals for weeks, months, or even years, combined with the explosive nature of CHIKV outbreaks and its demonstrated ability to quickly spread into new regions, has led to renewed interest in developing strategies for the prevention or treatment of CHIKV-induced disease. Therefore, this chapter briefly discusses the biology of CHIKV and the factors contributing to CHIKV dissemination, while also discussing the pathogenesis of CHIKV-induced disease and summarizing the status of efforts to develop safe and effective therapies and vaccines against CHIKV and related viruses.

CHIKUNGUNYA VIRUS EMERGENCE AND REEMERGENCE

Chikungunya virus (CHIKV) is believed to have originated in Africa and currently exists as three independent virus genotypes: West African, East/Central/South African (ECSA), and Asian. The first described incidence of human disease that was clearly attributable to CHIKV occurred on the Makonde Plateau of Tanzania (formerly Tanganyika) from October of 1952 until April of 1953 (1). The virus responsible for this first outbreak was isolated from the serum of a febrile patient and belonged to what was ultimately designated the ECSA genotype. While this is the first documented incidence of CHIKV, phylogenetic and retrospective analyses of clinical data suggest that the virus may have been present and causing disease much earlier. Many researchers believe that Chikungunya fever (CHIK) may have been incorrectly identified as dengue fever, due to some overlapping symptomatology, in multiple areas throughout Southeast Asia as early as the start of the 18th century (2, 3). During the initial outbreak in Tanzania, Lumsden noted appreciable numbers of Aedes aegypti mosquitoes in the huts of afflicted individuals, thereby providing initial evidence that the virus was vectored by mosquitoes (1). Subsequent studies suggested that in Africa CHIKV is maintained through an enzootic (sylvatic cycle) involving nonhuman primates and arboreal Aedes mosquitoes (4, 5). Spillover events occur when these CHIKV-infected, arboreal Aedes mosquitoes feed on naive humans and transmit the virus. If these infected individuals become viremic and are fed upon by urban A. aegypti mosquitoes, an urban transmission cycle involving human-to-mosquito-to-human transmission can be initiated, which can lead to significant disease out-
breaks. Since the initial outbreak in Tanzania, sporadic outbreaks of CHIK disease have continued to occur throughout Africa, including in Uganda, Malawi, and Nigeria (4, 5).

In 1958, a CHIKV outbreak was recognized in Bangkok, Thailand (6). Phylogenetic analysis of that outbreak virus demonstrated that the virus was distinct from the virus identified in Tanzania, and it was ultimately designated a separate Asian genotype, which is now endemic in Southeast Asia. Sporadic outbreaks of Asian genotype CHIKV have continued to occur throughout the region, including countries such as India, Vietnam, and Malaysia (7, 8). Unlike in Africa, evidence is lacking to support an enzootic cycle maintaining the Asian genotype virus in nature. Instead, Asian genotype CHIKV is believed to be maintained in an urban cycle between *Aedes* mosquitoes and naïve human hosts (9).

While cases of CHIK have been reported in Africa throughout the 20th century, a new strain of virus classified as Indian Ocean Lineage (IOL) reemerged in coastal Kenya in 2004 (10). The IOL strain of the virus quickly spread to Comoros and the Seychelles Islands before jumping to major population centers in the Indian Ocean region, including the Indian Subcontinent, where the virus was estimated to have caused over 1.5 million cases, and Sri Lanka (11–13).

Whole-genome and partial E1 sequences of numerous clinical isolates suggest that the IOL strain most likely evolved from a closely related ECSA strain (14). An important distinction was a single amino acid substitution in the E1 protein, A226V, which was found in the IOL virus isolates compared to ECSA CHIKV. Subsequent in vivo studies demonstrated that this change was necessary and sufficient for the virus to adapt to and efficiently utilize *Aedes albopictus* mosquitoes as a major vector of transmission (15). *Aedes aegypti* mosquitoes have historically been the primary vector for CHIKV transmission during other major outbreaks. However, the adaptation of IOL CHIKV to *Aedes albopictus* mosquitoes is viewed as a major factor which allowed the virus to reach epidemic levels in the Indian Ocean region, especially in areas where *A. albopictus* mosquitoes were the dominant mosquito species. Importantly, this expanded vector range also had implications for CHIKV’s subsequent introduction and spread into temperate areas, such as Italy, where *A. albopictus* was the vector responsible for local transmission (16, 17).

Historically, CHIKV has been a public health threat contained to the Eastern Hemisphere. However, the rapid spread of CHIKV throughout the Indian Ocean region, as well as its emergence in Italy and France, combined with the broad distribution of *Aedes* mosquitoes in both North and South America, raised concern that CHIKV might emerge in the Western Hemisphere (16, 17). Despite frequent incidents of CHIKV-infected individuals traveling into the Western Hemisphere, including a number of viremic travelers entering the United States (reviewed in reference 18), there were no documented cases of localized CHIKV transmission in the Americas during the height of the CHIKV outbreak in the Indian Ocean region (18). However, thoughts that CHIKV might not be capable of establishing infection in the Americas were disproved by an outbreak of CHIKV disease in late 2013, when the first reported cases of human-to-human transmission of CHIKV-induced disease appeared in the French region of the island of St. Martin in the Caribbean (19). These marked the first documented occurrence of non-traveler-associated CHIKV in the Western Hemisphere. Molecular and phylogenetic studies of viruses isolated from the outbreak have identified them as of the Asian genotype, most closely related to those circulating in the Philippines, China, and Micronesia prior to the Caribbean outbreak (20, 21). The Caribbean strain of CHIKV quickly disseminated from St. Martin to other island nations of the Greater and Lesser Antilles, including the Virgin Islands, Aruba, and Barbados (22). By early 2014, cases of the virus were detected in mainland South America in France Guiana, where local spread occurred there and into neighboring Guyana. Furthermore, limited localized transmission also occurred in Florida, with 12 reported cases of localized transmission (CDC ArboNet) (23). The Caribbean outbreak CHIKV has since caused local disease in Puerto Rico, the Dominican Republic, Colombia, and Mexico (Pan American Health Organization), with well over 1 million cases of CHIKV-induced disease occurring in the America’s since CHIKV’s introduction in 2013 (Pan American Health Organization). Importantly, CHIKV continues to cause disease in countries throughout South and Central America and therefore still has the ability to move into new areas, including the United States.

The recent CHIKV outbreaks in the Indian Ocean region, Southeast Asia, the South Pacific, and the Americas illustrate the importance of several factors in promoting CHIKV transmission. One of the major factors is the increased level of air travel, which almost certainly promotes the spread of CHIKV into new areas. During the height of the Indian Ocean outbreak, a number of CHIKV-infected travelers, who were documented to be viremic and thereby capable of transmitting the virus to permissive mosquitoes, entered the United States (18),
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and while no outbreak within the United States can be attributed to these individuals, it is likely that much, if not all, of the spread of CHIKV into other areas of the world was mediated by infected travelers (18). Furthermore, the localized transmission of the Caribbean strain of CHIKV observed in Florida in 2014 likely resulted from introduction of the virus by an infected traveler. A second major factor is the distribution of permissive mosquito vectors. Both A. aegypti and A. albopictus are capable of transmitting CHIKV, and the broad distribution of these mosquito vectors has certainly contributed to the expansion of CHIKV outbreaks in a manner similar to the global circulation of dengue virus and the more recent introduction and spread of Zika virus (24, 25). Lastly, changes in the virus itself have also contributed to the virus’s ability to cause widespread outbreaks. As noted above, a mutation in the IOL strain of CHIKV resulted in an expansion of the virus’s host range to A. albopictus mosquitoes, an event which allowed the virus to be spread in more temperate areas where A. aegypti mosquitoes are not found (26). Importantly, unlike the ECSA CHIKV strains, viruses of the Asian CHIKV genotype, which is the type that was introduced into the Caribbean, are less able to adopt the enhanced transmission phenotype in the A. albopictus mosquito vector (24, 27). In vivo studies of A. albopictus mosquitoes infected with engineered mutants of Asian CHIKV indicated that two independent amino acid changes (T98A and E226V in E1), which have yet to be observed in Asian genotype viruses found in nature, are required for the virus to efficiently adapt to use A. albopictus mosquitoes (27). Further findings from this study suggest that the acquisition of these separate mutations by Asian genotype viruses is unlikely due to intrinsic evolutionary constraints. This makes it less likely that the Caribbean strain of CHIKV will be capable of efficiently adapting to A. albopictus mosquitoes, which might limit the virus’s ability to be spread in temperate areas within the United States or other parts of the Americas. However, this might be complicated by the fact that in 2014, a strain of CHIKV belonging to the ECSA genotype reemerged in Brazil and has caused significant disease in numerous regions of the country (28). This outbreak marks the first time an ECSA genotype strain of CHIKV has been found in the Western Hemisphere associated with documented cases of local transmission. Abundant A. aegypti mosquitoes present in this environment have most likely fueled the outbreak, and currently, there is no evidence to suggest that this virus has mutated to adapt to A. albopictus mosquitoes, similar to what was observed with IOL CHIKV. Nevertheless, this virus still has the potential to gain a single adaptive mutation which could ultimately lead to vector expansion and movement of the virus into new regions of the world, including the United States.

CHIKV DISEASE

Most patients suffering from acute CHIKV disease present with high fever and arthralgia, as illustrated by a study by Thiberville et al. in which 100% of outpatients suffering from acute CHIKV infection during the Reunion Island epidemic presented with high fever and arthralgia (29). CHIKV-induced arthritides are often debilitating, and the name chikungunya, which is derived from the Makonde language, translates as “that which bends up,” describing the posture taken by persons suffering from CHIKV-induced disease (1). Acute CHIKV-induced arthralgia resolves over a period of several days to weeks; however, arthralgia can persist in some individuals for months to years. In addition to fever and arthralgia, common symptoms of acute CHIKV infection include asthenia, myalgia, and headache (reviewed in reference 30), while other symptoms, such as maculopapular rash and nausea, are also frequently observed in CHIKV patients. Patients frequently present with lymphopenia, while elevated C-reactive protein, elevated liver enzymes, and signs of thrombocytopenia are also observed in a subset of patients with acute CHIKV infection (30). Although the case fatality rate for CHIKV is extremely low, the recent outbreaks have seen a rise in atypical disease manifestations, including encephalitis in infants and multiorgan failure and mortality in elderly individuals or persons with underlying medical conditions (reviewed in reference 30).

CHIKV-Induced Joint and Muscle Disease

Polyarthritis and myalgia are common attributes of many viral infections; however, severe incapacitating arthralgia is the most prominent feature of acute CHIKV infection (reviewed in reference 30). Following CHIKV infection, patients often rapidly present with sudden onset of severe fever, arthralgia, and myalgia (29, 31). However, while other symptoms, such as fever, resolve within a few days, arthralgia resolves over a longer period, a disease attribute that distinguishes CHIKV-induced arthralgia from that induced by viruses such as dengue virus. This is illustrated by a study by Thiberville et al. in which CHIKV-induced fever had resolved by day 7 after their first medical visit in 100% of patients (n = 54), while approximately 65% of patients still
reported joint pain 25 days after their initial doctor’s visit (29). Furthermore, as noted below, a significant fraction of individuals complain of persistent arthralgia for months to sometimes years after onset (32). During the acute phase of the disease, arthralgia is usually symmetrical and affects multiple joints, commonly the joints of the toes, fingers, ankles, wrists, knees, and elbows (29, 31, 33). Although overt signs of infiltration are evident in a small subset of affected individuals, acute CHIKV-induced joint disease is generally not erosive, and swelling around the joints is a common feature of acute CHIKV disease (34); however, previously damaged joints may predispose individuals to increased risk of prolonged arthralgia (35).

As noted above, debilitating acute arthralgia is the defining symptom of CHIKV-induced disease, and although CHIKV-induced joint pain is generally most severe at early times post onset, resolution of acute arthralgia can often occur over a period of several weeks. In a subset of infected individuals, symptoms fail to resolve for periods ranging from several months to years after the initial onset of disease, and this joint pain and stiffness can have a significant impact on quality of life (32, 35). The fraction of persons suffering from persistent CHIKV was historically considered to be low, as illustrated by a study by Brighton and Simson that found that 12% of CHIKV patients had persistent symptoms up to 3 years postonset (36). However, during the recent outbreak on Reunion Island, chronic disease appeared to be more prevalent, with 57% of subjects reporting persistence or episodes of recurrence in one study (37), while a second study found 26% of patients reporting residual arthralgia in multiple joints at day 300 after disease onset (29). Furthermore, although recurrent joint stiffness and pain appear to be the major manifestations of chronic CHIKV arthralgia, there have been reports of more severe joint disease in persons suffering from persistent CHIKV-induced arthritides, including erosive arthritis (32, 36, 38). Although the factors that contribute to chronic CHIKV-induced arthralgia are poorly understood, it is clear that increased age, higher viral loads, and the severity of the acute phase of infection are major risk factor for developing persistent disease. Several studies have found that people over the age of 45 are more likely to develop long-term joint pain and stiffness (29, 32, 37, 39), while Thiberville et al. found that persons with ongoing joint pain at 300 days postinfection presented with a higher number of affected joints during the acute stage of the disease (29).

The pathogenesis of acute and chronic CHIKV-induced arthralgia is not completely understood; however, there is strong evidence of CHIKV replication in affected tissues. Furthermore, a growing body of evidence suggests that viral replication within joint tissues elicits an overactive host inflammatory response, which then drives the development of joint pathology and arthralgia. Biopsy results from patients suffering from CHIKV-induced myositis provided evidence that CHIKV can replicate in muscle cells, and this was further confirmed in primary culture studies demonstrating that muscle satellite cells are capable of supporting CHIKV replication (40). Although there is little direct evidence for CHIKV replication within the joints of affected patients due to a lack of synovial biopsy samples from patients with acute CHIKV infection, CHIKV has been shown to replicate efficiently in human synovial fibroblasts (41), and studies from mouse and nonhuman primate models have demonstrated that synovial joints are a major target of CHIKV replication in vivo (42–45).

There is also evidence suggesting that persistent viral replication may contribute to chronic CHIKV disease. One study, by Hoarau et al., found evidence for persistent CHIKV replication in a single patient suffering from chronic CHIKV arthralgia (46); however, considering the limited sample size in this study, additional studies are needed to confirm these results.

Given the difficulty in obtaining synovial biopsies that span the acute to chronic disease stages from CHIKV-infected humans, a number of groups have turned to animal models to study chronic CHIKV disease. Studies with cynomolgus macaques found that replication was detectable in lymphoid tissues for up to 3 months postinfection (47). Likewise, experiments with mouse models have found detectable levels of CHIKV RNA in joint tissues for up to 4 months postinfection (48), although infectious virus has not yet been detected in these systems. These results all suggest that CHIKV can persist in individuals for long periods, even in the face of a potent antiviral immune response. However, the nature of this persistence and whether persistent viral replication drives long-term chronic joint disease in humans remain to be determined.

Although direct viral replication within joint tissues is thought to contribute to acute CHIKV-induced joint disease, and possibly chronic disease, there is also evidence suggesting that aspects of the host inflammatory response contribute to disease pathogenesis. While components of the innate and adaptive immune response, such as the type I interferon (IFN) system and antiviral antibody, contribute to CHIKV control and clearance (reviewed in reference 49), a significant body of evidence suggests that overactive inflammatory responses clearly
contribute to the pathogenesis of acute CHIKV-induced arthralgia and swelling. The severity of CHIKV-induced arthralgia is associated with increased levels of a number of proinflammatory cytokines within the serum of infected humans (50, 51), and inflammatory cell infiltration into joint tissues is a prominent feature in CHIKV-infected mice and nonhuman primates (42, 44, 45, 52), which suggests that aspects of the host inflammatory response contribute to CHIKV-induced joint disease. This is further supported by mouse studies in which depletion of monocytes reduced the severity of CHIKV-induced arthritis (44). Mouse studies also suggest that components of the adaptive immune response modulate the severity of CHIKV-induced arthritis, with CD4 T cells contributing to CHIKV-induced joint swelling (53). Therefore, careful targeting of specific immune components that promote disease may represent a therapeutic avenue in the treatment of CHIKV-induced disease.

**Neurologic Involvement**

Unlike in the case of encephalitic alphaviruses, such as Eastern and Venezuelan equine encephalitis viruses, neurologic disease is not usually associated with CHIKV. However, a small subset of adult patients requiring hospitalization exhibited signs of syndromes such as acute flaccid paralysis, Guillain-Barré syndrome, and encephalopathy (54–56). This is illustrated by a study during the Reunion Island CHIKV epidemic in which 25% of patients with atypical CHIKV infection reported neurologic involvement, including malaise and meningoencephalitis (57). Children are also at risk of developing neurologic complications, where vertical mother-to-child transmission puts newborns at significant risk of developing encephalopathy that can result in lifelong neurologic consequences (58–60).

**Mortality Associated with CHIKV Infection**

CHIKV-induced mortality, although rare, does occur, with an approximate case fatality rate of 1 in 1,000 (reviewed in reference 30). Very young (e.g., neonates) and elderly individuals, as well as people with underlying medical conditions, comprise the majority of these cases, with causes of death ranging from encephalitis to hepatitis and multiple-organ failure (30).

**Other Clinical Manifestations**

A transient maculopapular rash on the thorax and the medial aspects of the limbs during CHIKV disease is common; however, a small subset of individuals do develop more severe skin manifestations, including ulcers and vasculitis (reviewed in reference 30). Although the pathogenesis of CHIKV-induced skin disease is poorly understood, active viral replication within the skin might contribute to CHIKV-induced skin disease. Other rare but potentially serious manifestations of CHIKV disease include ocular disease, including uveitis (61). Lastly, fatigue is a common complaint associated with CHIKV infection and may persist for months to years in some individuals (62).

**CHIKV VACCINES AND THERAPEUTICS**

The reemergence of CHIKV, with subsequent spread in the Indian Ocean region and its introduction into the South Pacific and the Americas, has rekindled interest in the development of vaccines for the prevention of CHIKV infection and therapeutics for treating acute and chronic CHIKV-induced disease. Unfortunately, there are currently no approved CHIKV vaccines or therapies, and despite the scope of the current CHIKV epidemic, treatment options for CHIKV-induced disease are generally limited to palliative care using nonsteroidal anti-inflammatory drugs (NSAIDs) and hydration. Given the scope of the recent CHIKV outbreaks and the limited treatment and prevention options available, significant effort has been put into developing new vaccine and therapeutic pipelines for CHIKV, and we briefly summarize current progress in both of these areas.

**CHIKV Vaccines**

Although CHIKV is a threat to spread within developed countries, such as the United States and European countries, developing countries have borne the brunt of CHIKV-induced disease over the past 10 years and are at greatest risk of continued CHIKV spread. Therefore, for a CHIKV vaccine to be useful in these areas, it would need to be relatively inexpensive to manufacture and administer and preferably be highly immunogenic after a single dose, while having no to minimal side effects. Furthermore, since older individuals are at increased risk of developing chronic CHIKV-induced arthralgia and for severe CHIKV-induced disease (29, 32, 37, 39), a successful vaccine would ideally be safe and immunogenic in this population. While multiple vaccine strategies have been explored in preclinical studies (reviewed in depth in reference 63), to date, four vaccines have entered human trials and many vaccines are in differing stages of preclinical testing (Table 1). For the purposes of this review, we focus on vaccines that have entered into clinical trials while briefly discussing other vaccine strategies.
CHIKV vaccine research dates back 40 years, with much of the early CHIKV vaccine work focusing on traditional inactivated and or cell culture-adapted live attenuated vaccines. Initial attempts at generating a CHIKV vaccine focused on using formalin-inactivated virus derived from the African 167 CHIKV strain, which was produced from green monkey kidney cells, chicken embryo cells, or concentrated suspension cultures (64). This inactivated vaccine was initially tested by intraperitoneal inoculation into 3- to 4-week-old Swiss Bragg mice with a prime-boost, 2-dose schedule, followed by intracerebral challenge. The vaccine exhibited good efficacy in the mouse model and was later tested for efficacy in humans, in whom it was found to elicit a neutralizing antibody response with no adverse events (65).

The second vaccine that was tested in humans was developed at the Walter Reed Army Institute of Research, where Levitt et al. set out to increase the efficacy of the green monkey kidney-based inactivated vaccine by generating a live attenuated vaccine. A human CHIKV isolate from a 1962 Thai outbreak, strain 15561, was plaque purified and passaged 18 times in human embryonic lung MRC-5 cells (66). On the 18th passage, three plaque-purified clones, clones 25 to 27, which exhibited a uniform plaque morphology, underwent safety testing by intracranial inoculation into neonatal (1- to 3-day-old) mice. In contrast to the parental virus, which caused 61% mortality, none of the three passaged viral isolates caused mortality. In subsequent efficacy studies, clone 25 exhibited 100% protection against lethal CHIKV challenge in weanling mice, and this virus was designated 181/25. Following the successful testing in mice, the 181/25 vaccine was then taken forward for additional evaluation in nonhuman primates (66). In a dose escalation experiment in which the vaccine was administered at doses ranging from 3.5 to 5.5 log_{10} PFU, vaccinated animals exhibited complete protection from CHIKV viremia following challenge (66).

Following the mouse and nonhuman primate studies, the 181/25 vaccine was tested for virulence in humans in a phase I clinical trial. In this trial, involving 15 people, there were no adverse events reported and no conclusive evidence of a difference between the naive group receiving either the 181/25 vaccine or a mock vaccination (67). Therefore, 181/25 was considered avirulent in humans and proceeded to phase II trials, where the 181/25 vaccine (now called TSI-GSD-218) was evaluated in a double-blind 73-person efficacy trial (68). Following intramuscular injection with 0.5 log_{10} PFU of vaccine (n = 59) or a mock vaccine (n = 14), subjects were interviewed to discuss symptoms on days 1 to 4, 10, 14, and 28 postvaccination. The group that received the vaccine developed neutralizing antibodies in 98.3% of cases by 28 days postvaccination, with 85% of the vaccinees remaining seropositive 1 year later (68). Some members of both the experimental and control groups experienced symptoms at the site of inoculation and flu-like symptoms, with no statistically significant difference between the groups. However, 5 of the 59 patients that received the vaccine developed transient unilateral arthralgia in 1 or 2 of their joints, compared to 0 cases in the control group, which led the TSI-GSD-218 vaccine to be abandoned following the phase II trial (68).

Although development of the 181/25 vaccine was halted in phase II trials, with the reemergence of CHIKV and subsequent large-scale epidemics, there has been some interest in revisiting this vaccine (69). However, recent work has illustrated potential pitfalls associated with this vaccine, including spread into mosquito species

### Table 1: CHIKV vaccine strategies

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Platform</th>
<th>Dosing scheme</th>
<th>Stage of development</th>
<th>Year of original description</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td>167, inactivated</td>
<td>Inactivated</td>
<td>Multidose</td>
<td>Phase I</td>
<td>1971</td>
<td>64, 65</td>
</tr>
<tr>
<td>181/25</td>
<td>Live attenuated</td>
<td>Single dose</td>
<td>Phase II</td>
<td>1986</td>
<td>66 - 68, 70, 71, 86</td>
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<tr>
<td>Consensus capsid DNA</td>
<td>DNA</td>
<td>Multidose</td>
<td>Preclinical</td>
<td>2008</td>
<td>86</td>
</tr>
<tr>
<td>ECSA based, inactivated</td>
<td>Inactivated</td>
<td>Multidose</td>
<td>Preclinical</td>
<td>2009</td>
<td>83</td>
</tr>
<tr>
<td>VSV/CHIKV VLP</td>
<td>VLP</td>
<td>Multidose</td>
<td>Phase I</td>
<td>2010</td>
<td>79, 80</td>
</tr>
<tr>
<td>Structural gene DNA</td>
<td>DNA</td>
<td>Multidose</td>
<td>Preclinical</td>
<td>2011</td>
<td>87</td>
</tr>
<tr>
<td>Adenovirus chimera</td>
<td>Chimeric</td>
<td>Single dose</td>
<td>Preclinical</td>
<td>2011</td>
<td>85</td>
</tr>
<tr>
<td>Insect cell VLP</td>
<td>VLP</td>
<td>Single dose</td>
<td>Preclinical</td>
<td>2011</td>
<td>89, 90</td>
</tr>
<tr>
<td>CHIKV/IRES</td>
<td>Live attenuated</td>
<td>Single dose</td>
<td>Preclinical</td>
<td>2011</td>
<td>74-78</td>
</tr>
<tr>
<td>Alphavirus chimera</td>
<td>Chimeric</td>
<td>Single dose</td>
<td>Preclinical</td>
<td>2011</td>
<td>84</td>
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<tr>
<td>E2 protein</td>
<td>Subunit</td>
<td>Multidose</td>
<td>Preclinical</td>
<td>2012</td>
<td>88</td>
</tr>
<tr>
<td>MV/CHIKV VLP</td>
<td>VLP</td>
<td>Multidose</td>
<td>Phase I</td>
<td>2013</td>
<td>81, 82</td>
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</table>
The capacity for 181/25 vaccine could revert to a virulent phenotype after 5 serial mouse brain passages in neonatal type I IFN receptor−/− (IFNAR−/−) mice. This reversion to virulence was caused by both direct revertants of the previously identified residue 82 mutation and by other loss of positive-charge, surface-exposed mutations near residue 82 of the E2 protein (74). This work strongly suggested that more stable attenuation strategies were needed for developing safe live attenuated CHIKV vaccines.

Another promising live attenuated vaccine that has been extensively studied in the preclinical stage is the CHIKV/IRES vaccine produced by Plante et al. (75). By utilizing an encephalomyocarditis virus internal ribosomal entry site (IRES) that is incapable of translation in arthropod cells, they were able to produce an immunogenic and attenuated vaccine which was incapable of growing in the mosquito vector. This vaccine was found to be efficacious and safe in multiple mouse models and nonhuman primates (75, 76). The CHIKV/IRES vaccine was further tested for safety and stability by trying to mutate the virus in a worse-case-scenario serial mouse brain passage experiment. The IRES-based vaccine remained attenuated, while the 181/25 vaccine, which was run in parallel as a control, became neurovirulent, leading to fatal outcomes in (IFNAR−/−) mice (74). The vaccine was also successfully tested for its ability to protect against a closely related virus in the Semliki Forest clade, o’nyong-nyong virus (77). It was further shown that the neutralizing antibody response elicited by CHIKV/IRES in mice was significant and sufficient to elicit full protection against a lethal challenge (78).

After CHIKV’s reemergence, the first new vaccine strategy to go forward for testing in humans was a virus-like particle (VLP) vaccine produced and tested by Akahata et al. (79). The VLPs were produced in HEK293T cells using CHIKV glycoproteins derived from the 37997 CHIKV strain in a lentiviral vector along with the vesicular stomatitis virus G protein. A three-dose vaccination regimen in nonhuman primates elicited neutralizing antibodies which when passively transferred to mice were protective against lethal CHIKV challenge (79). The vaccine has been evaluated in a phase I dose escalation trial (80) in which three different doses, 10 μg, 20 μg, and 40 μg, were administered three times over a period of 20 weeks in cohorts of 5, 10, and 8 people. All three doses elicited a strong neutralizing antibody response and were well tolerated by the subjects (80).

The most recent vaccine candidate to have been evaluated in human trials is a measles virus (MV)-vectored VLP produced by Brandler et al. (81). This vaccine uses a live attenuated Schwarz MV as a vector for CHIKV structural proteins from the La Reunion (OPY2006) strain. This recombinant virus (MV-CHIKV) was tested in type I IFN receptor-deficient (IFNAR−/−) mice transgenic for the human CD46 MV receptor that are capable of supporting MV replication. Three doses (3 log10 PFU, 4 log10 PFU, and 5 log10 PFU) were tested by inoculating mice twice over a 1-month interval. All three doses elicited neutralizing anti-CHIKV antibody responses; the low dose was found to be 80% efficacious, and the higher two doses were 100% protective against a lethal challenge. This vaccine was then subjected to a randomized, double-blind, placebo-controlled, phase I clinical trial (82). Doses of either 1.5 × 10^4 PFU, 7.5 × 10^4 PFU, or 3.0 × 10^5 PFU were administered to 12 subjects of each cohort, and a negative control cohort of 6 people received a Priorix measles-mumps-rubella vaccine, with each cohort receiving a total of three inoculations on days 0, 28, and 90. The vaccine did elicit neutralizing antibody in a dose-dependent manner, and importantly, previous measles vaccination did not adversely affect the vaccine’s ability to elicit CHIKV-specific immune responses. The vaccine was also relatively well tolerated at the lower two doses, with most adverse events being classified as mild or moderate. However, 58% of the individuals in the cohort with 3.0 × 10^5 PFU did exhibit adverse events, including flu-like illness, pain at the site of injection, and dispensed but transient myalgia (82).

A multitude of other CHIKV vaccine candidates have been produced and are in different stages of preclinical testing. An inactivated vaccine was produced and tested by Tiwari et al., utilizing a Vero cell-adapted ECSA strain of CHIKV, and proved capable of producing neutralizing antibodies (83). Several chimeric viruses were tested, utilizing the either alphavirus or adenovirus vectors with the structural genes of CHIKV (84,
These vaccines were capable of eliciting a neutralizing antibody response and protecting mice from a virulent challenge. Two DNA-based vaccines were also produced and were also found to protect mice and, in nonhuman primates, were found to induce a robust immune response (86, 87). Other vaccine strategies were attempted, such as a series of subunit vaccines (88, 89) and a VLP-based vaccine produced from insect cells (90). These strategies also were found to have their strengths.

As previously stated, a vaccine for CHIKV should exhibit multiple traits for effective use. These traits would be slightly different for a traveler’s vaccine compared to one intended for implementation in the regions where this virus is endemic. A traveler’s vaccine could be a multidose and expensive and convey only short-lived protection. However, since this virus disproportionately affects equatorial developing countries, cost and efficacy take on added weight when considering utility. The vaccine would have to be manufactured quickly, and at low cost, to be implemented in the large and relatively poor populations most at risk. The vaccine should elicit a strong and long-lived immune response and do so with a single dose. A multidose vaccine may prove ineffective if the patient either chooses not to return or is incapable of coming in for subsequent booster vaccinations. The vaccine would have to be safe and easy to administer due to the lack of advanced health care in some of the regions where the virus is endemic. Another important trait of any live attenuated vaccine is that it proves stable in its nonvirulent phenotype and is not going to be accidentally spread by the mosquito vectors. Though this is not an issue in other vaccine platforms, the live attenuated vaccine strategy is thought to be the best option for a virus that so heavily impacts developing countries.

Antivirals against CHIKV

As noted above, although there are multiple CHIKV vaccines in various stages of preclinical and clinical development, there are currently no vaccines approved for use in humans. The situation with antiviral therapies is similar, in that while a number of antiviral strategies are being pursued, currently approved treatments for acute CHIKV infection are limited to NSAIDs. How-

TABLE 2

<table>
<thead>
<tr>
<th>Antiviral</th>
<th>Target</th>
<th>FDA approval no.*</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>Ribavirin</td>
<td>Virus</td>
<td>018859</td>
<td>92, 93</td>
</tr>
<tr>
<td>Mycophenolic acid</td>
<td>Virus</td>
<td>050791</td>
<td>94</td>
</tr>
<tr>
<td>6-Azauridine</td>
<td>Virus</td>
<td></td>
<td>93</td>
</tr>
<tr>
<td>Homoharringtonine</td>
<td>Virus</td>
<td>203585</td>
<td>97</td>
</tr>
<tr>
<td>(omacetaxine mepesuccinate)</td>
<td></td>
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<tr>
<td>Polyclonal antibodies</td>
<td>Virus</td>
<td></td>
<td>79, 98</td>
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<td>Mabs</td>
<td>Virus</td>
<td>006002</td>
<td>36, 106, 108, 109</td>
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<td>Chloroquine</td>
<td>Host</td>
<td></td>
<td>112, 115</td>
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<tr>
<td>Bindarit</td>
<td>Host</td>
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Antiviral Target

Ribavirin Virus 018859 92, 93
Mycophenolic acid Virus 050791 94
6-Azauridine Virus 93
Homoharringtonine (omacetaxine mepesuccinate) Virus 203585 97
Polyclonal antibodies Virus 79, 98
Mabs Virus 43, 99, 100, 104
Chloroquine Host 36, 106, 108, 109
Bindarit Host 112, 115

*Reflects approval for applications other than CHIKV. All numbers reflect NDAs.
virin has been shown to have antiviral activity against CHIKV both in vitro and in a small clinical study (92, 93), which suggests that ribavirin may have utility in treating CHIKV cases.

Several other promising antiviral compounds include mycophenolic acid, 6-azauridine, and harringtonine. Similar to ribavirin, mycophenolic acid acts to inhibit inosine monophosphate dehydrogenase, reduces cellular GTP pools, and therefore exhibits broad-spectrum antiviral activity. In vitro studies have shown that mycophenolic acid protects cells against CHIKV-induced apoptosis and reduces viral yields from treated cells (94). The compound 6-azauridine is a uridine nucleoside analogue which inhibits the enzyme orotidine monophosphate decarboxylase, required for the synthesis of pyrimidines. Inhibition of pyrimidine synthesis leads to reduced UTP levels, and therefore, 6-azauridine has antiviral activity against a number of DNA and RNA viruses, including CHIKV, with which the drug shows strong inhibition of replication in vitro (93). Harringtonine and its derivative homoharringtonine are natural plant alkaloids which inhibit protein synthesis in eukaryotic cells. Synthetic homoharringtonine, renamed omacetaxine mesylate, received FDA approval for the treatment of chronic myelogenous leukemia (FDA NDA 203585). It is believed to function by stalling host translation by competing with tRNAs at the ribosome and can also halt the cell cycle (95, 96). Recently, harringtonine has been shown to effectively inhibit CHIKV protein synthesis in vitro at low 50% effective concentrations (97); however, it has not been tested for efficacy in vivo.

Antibody therapies
Anti-CHIKV antibody has long been known to be a correlate of CHIKV vaccine-mediated protection, and a number of passive-immunization studies have shown that CHIKV-specific neutralizing antibodies can protect animals from CHIKV replication and disease (79, 98). Therefore, CHIKV antibodies have been evaluated as both prophylactic and postexposure therapies for the treatment of acute CHIKV disease in patient populations, and a number of antibody formulations are at various stages of preclinical development. Couderc et al. were able to demonstrate protection against CHIKV disease in both neonatal and IFN receptor knockout mice (IFNAR−/−) through passive transfer of human donor convalescent-phase plasma (98). In this study, protection from disease was achieved when antibodies were administered within the first 24 h of infection. Akahata et al. have also found that nonhuman primate polyclonal antibodies directed against CHIKV VLPs protected IFNAR−/− mice against disease (79).

In addition to polyclonal-antibody studies, both human and murine monoclonal antibodies (MAbs) directed at the E1 and E2 structural glycoproteins have also been identified that neutralize virus in vitro and are protective in mice (99, 100). Many of the E2 antibodies target diverse regions of the protein to neutralize virus. Generally, clinical improvements in mice are observed when MAb treatment is started within the first 24 h following infection (101–103). In addition to single-MAb therapies, other investigators have developed combinatorial-MAb therapies to help prevent against neutralization escape variants (43). Pal et al. recently demonstrated that genetically engineered escape mutants of CHIKV with resistance to neutralization by two independent MAbs were mildly attenuated in mice and failed to revert to wild type in both mosquitoes and mice (104). The combination of studies on polyclonal antibody and MAb therapies against CHIKV suggest that they have strong potential for use in humans at higher risk for disease, when treated early in infection. However, it remains unclear whether antibody therapy would be useful in general populations during CHIKV outbreaks due to costs and logistical concerns around antibody delivery. However, anti-CHIKV antibody therapies might be very useful in specific at-risk populations, such as laboratory workers suffering from known virus exposures, immunosuppressed individuals, or CHIKV-infected women during the late stage of pregnancy (60, 105). Of particular note, given that mother-to-child CHIKV transmission during childbirth puts infants at increased risk of developing CHIKV-induced neurologic disease, which can result in sequelae with lifelong consequences, anti-CHIKV antibodies represent a promising approach for protecting this population. This could take the form of administering antibodies to women in the latter stages of pregnancy who have active CHIKV infections or reside in an active outbreak locality, as a means of reducing CHIKV viremia and thereby limiting chances of transmission to the infant, or by direct administration of antibody to the neonate.

Host-targeted antivirals
Clinical trials with host targeted antivirals have focused on chloroquine, a class of 4-aminoquinolone drug discovered in 1934 and which was originally used as an antimalarial drug (FDA NDA 006002). Chloroquine has also been demonstrated to have potent antiviral activity against CHIKV in vitro. Mechanistic studies suggest that the drug increases endosomal pH, thus
preventing the low-pH fusion of the E1 protein during early entry of the virus (106). While Khan et al. were able to demonstrate efficacy of the drug when used preinfection, during infection, and postinfection at micromolar concentrations in vitro, it was shown to be ineffective when used greater than 3 h postinfection. An early report from Brighton found that chloroquine treatment improved chronic CHIKV-associated joint symptoms in 50% of a cohort of patients (107). However, this was an open study with a small number of patients. Importantly, in follow-up studies, chloroquine was found to be ineffective during two separate human clinical trials conducted in India and Reunion Island (108, 109). In a study conducted by de Lamballerie et al., patients who received chloroquine complained of more frequent arthralgia than those that received placebo by day 200 of treatment (109). The early mode of action of chloroquine coupled with its ineffectiveness in several clinical studies suggests that it may have limited potential for treatment of acute human CHIK disease.

As noted above, there is a significant body of evidence which suggests that the host inflammatory response contributes to the pathogenesis of CHIKV-induced arthritides. Therefore, therapies that inhibit aspects of the host inflammatory response also hold promise in the treatment of CHIKV-induced disease. Bindarit, a small-molecule inhibitor of monocyte chemotactic protein 1 (MCP-1) synthesis, has shown potent anti-inflammatory actions against cancer-induced inflammation and autoimmune inflammation in several rodent models (110, 111). In mouse models of CHIKV pathogenesis, macrophage numbers and MCP-1 levels have been tightly associated with joint inflammation, arthritis, and myositis. Chen et al. demonstrated that intraperitoneal administration of bindarit twice a day from the day of infection resulted in significant reductions in symptoms and the duration of disease in mice, which was independent of viral loads in affected tissues (112). However, Poo et al. have recently shown that mice deficient for the cognate receptor of MCP-1, CCR2, have prolonged and more severe symptoms and inflammation than wild-type mice challenged with CHIKV (113). Notably, cellular inflammation in CCR2−/− mice is dominated by neutrophils and, later, eosinophils, as opposed to the classical monocyte/macrophage response seen in wild-type mice. Based upon these conflicting results, the use of bindarit to treat CHIKV in humans would require additional small-animal model studies to clarify the mode of action of the drug, while additional the safety and efficacy tests in other models, such as nonhuman primate models of CHIKV infection, are likely warranted.

**Therapies for chronic CHIKV disease**

As noted above, a significant fraction of CHIKV-infected individuals suffer from chronic joint pain and stiffness for months or even years postinfection. Given the duration of these symptoms and the fact that they cause a significant decrease in quality of life (30), there is a clear need for effective therapies for treating chronic CHIKV-associated joint pain. Unfortunately, the development of effective therapies is hampered by the fact that the pathogenesis of chronic CHIKV-associated joint pain is poorly understood. For example, although there are limited data suggesting that the CHIKV persistence within joints may contribute to chronic disease (46), it is unclear whether drugs that inhibit CHIKV replication will have any benefit if administered during the chronic stage of disease. Furthermore, there is evidence for ongoing inflammation in the joints of at least a subset of individuals suffering from the most severe aspects of chronic CHIKV-induced arthralgia, and evidence suggests that these individuals are likely to be helped by treatment with NSAIDs, as well as disease-modifying antirheumatic drugs, such as methotrexate (32, 114). However, it is unknown whether broad application of stronger anti-inflammatory and immunosuppressive therapies will be of benefit in treating chronic CHIKV disease, at which point in the disease process these strategies should be applied, or whether more specific anti-inflammatory drugs that target specific host pathways will be of benefit. Therefore, the development of more effective therapies or even approaches for safely using existing treatments, such as methotrexate, for treating chronic CHIKV disease is likely to require a much better understanding of the viral and host factors that drive disease pathogenesis.

**CONCLUSIONS**

The reemergence of CHIKV and its subsequent global spread illustrate how a combination of rapid global transit, broad mosquito vector distribution, and a lack of strategies for treating or controlling emerging pathogens can significantly impact public health, a scenario that is now being repeated with the emergence and spread of Zika virus in the Americas. In the case of CHIKV, the response to outbreaks over the past 12 years has provided important new insights into the pathogenesis of CHIKV disease, as well as strategies for developing new vaccines and therapies for treating acute and chronic CHIKV. However, additional work is needed in all of these areas both to deal with the ongoing CHIKV epidemic in the Americas and to prepare for future CHIKV outbreaks.
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