REVIEW ARTICLE

Edward W. Campion, M.D., *Editor*

Chikungunya Virus and the Global Spread of a Mosquito-Borne Disease

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CHIKUNGUNYA VIRUS AND CHIKUNGUNYA FEVER

Chikungunya virus is a mosquito-borne alphavirus; its name comes from a Makonde word describing the bent posture of persons with the severe arthralgia that is a hallmark of chikungunya fever, the disease caused by the virus.[1] Chikungunya virus was first isolated after a 1952–1953 epidemic in present-day Tanzania. Outbreaks were subsequently identified in Asia during the 1950s and 1960s. Like the related alphaviruses found in Australia and other parts of Oceania, as well as in South America, chikungunya virus causes an acute febrile illness that is typically accompanied by severe arthralgia. Alphaviruses have a single-stranded, positive-sense RNA genome approximately 11.5 kb in length that encodes four nonstructural proteins and three main structural proteins: the capsid and two envelope glycoproteins, E1 and E2, which form spikes on the virion surface (Fig. 1).[2,3] E2 binds to unknown cellular receptors to initiate cell entry through endocytosis, and E1 includes a fusion peptide, exposed at low pH in endosomes, which initiates the release of nucleocapsids into the host-cell cytoplasm.

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HISTORY AND ORIGINS OF CHIKUNGUNYA VIRUS

Chikungunya virus circulates in forested regions of sub-Saharan Africa in ancestral transmission cycles involving nonhuman primate hosts and arboreal mosquito vectors (Fig. 2).[4,5] Phylogenetic studies indicate that the establishment of the urban transmission cycle has occurred on multiple occasions from strains circulating in the eastern half of Africa in nonhuman primate hosts (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).[4] These instances of emergence and spread beyond Africa may have begun as early as the 18th century,[6] when sailing ships carried chikungunya virus along with humans and *Aedes aegypti* mosquitoes in numbers sufficient for circulation of the virus aboard ships, where stored water facilitated mosquito propagation. The first emergence of the virus into the urban cycle during the modern scientific era occurred between 1879 and 1956, when a member of the eastern, central, and southern African (ECSA) enzootic lineage was introduced into Asia (Fig. 2); currently available data on chikungunya virus strains and their sequences do not clarify whether this introduction into Asia occurred in the 19th century or more recently. This epidemic strain, called the Asian lineage, caused outbreaks in India and Southeast Asia and continues to circulate in the latter region.

In 2004, an outbreak involving another ECSA lineage progenitor began in coastal Kenya[7] before spreading to several Indian Ocean islands and to India, where it caused explosive epidemics involving millions of people.[8] Subsequently, infected

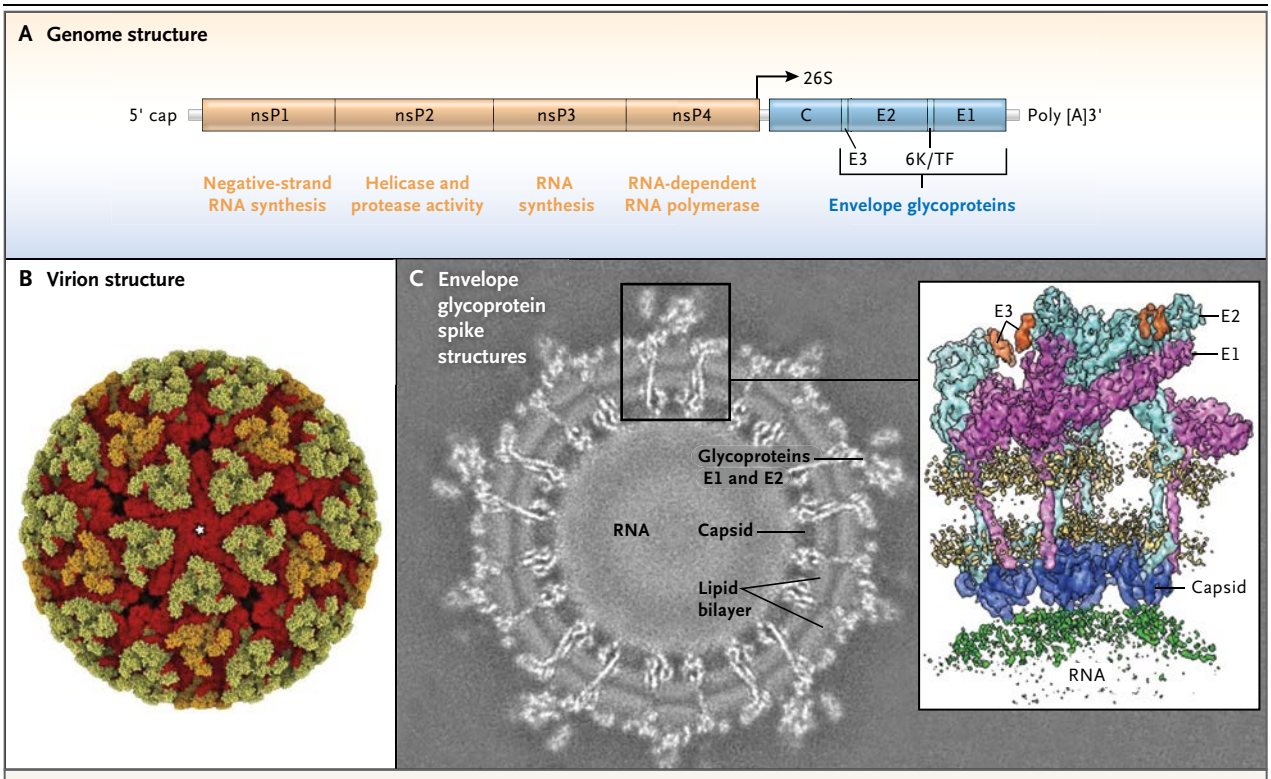

Figure 1. Chikungunya Virus Genetic and Physical Structure.

Panel A shows the organization of the chikungunya virus genome, including its nonstructural proteins 1 through 4 (nsP1–nsP4) and structural proteins C (capsid), E1–E3 (envelope glycoproteins), and 6K/TF (6K and TF [transframe] are alternative translation products of the same gene). Panel B shows the structure of the virion (image courtesy of Felix Rey, Institut Pasteur, Centre National de la Recherche Scientifique). Panel C shows spike-protein predicted structures based on atomic resolution structures of the envelope glycoproteins[2] and high-resolution cryoelectron microscopic reconstructions of chikungunya virus and other alphavirus particles.[3]

air travelers arrived in Europe, Asia, and the Americas, and local transmission ensued in Italy, metropolitan France, and many countries in South and Southeast Asia. The unprecedented magnitude of these outbreaks was probably influenced by several factors: increased air travel, which permitted rapid spread; the previous lack of exposure of human populations in the Indian Ocean basin and South Asia; further urbanization in most of the tropics, with denser human and urban mosquito populations; the invasion, since 1985, of *A. albopictus* (a mosquito that now serves as a second chikungunya virus vector in addition to *A. aegypti*) from its native Asia into islands in the Indian Ocean basin, Africa, and southern Europe, which was facilitated by increased global commerce; and a series of adaptive mutations in the new Indian Ocean lineage (IOL) chikungunya virus strains, which mediated enhanced virus transmission by *A. albopictus*.[9-13] This mosquito species was not implicated as a major vector in previous Asian epidemics, and the older Asian lineage is genetically constrained in its ability to adapt to this mosquito.[14]

Infected travelers did not initiate local transmission in the Americas during the peak of the 2006–2009 IOL-strain outbreaks, despite many cases having been imported.[15] However, an Asian-lineage chikungunya virus strain was introduced into the island of St. Martin in October 2013[16] and subsequently spread throughout the Caribbean and Central America as well as into northern South America and Florida, where 11 locally acquired cases have occurred. It seems likely that there will be further spread throughout the Americas, where tens of millions of previously unexposed persons are at risk and both chikungunya virus vectors are widespread (Fig. 2), as well as in Polynesia, which is a site of current epidemics.[17]

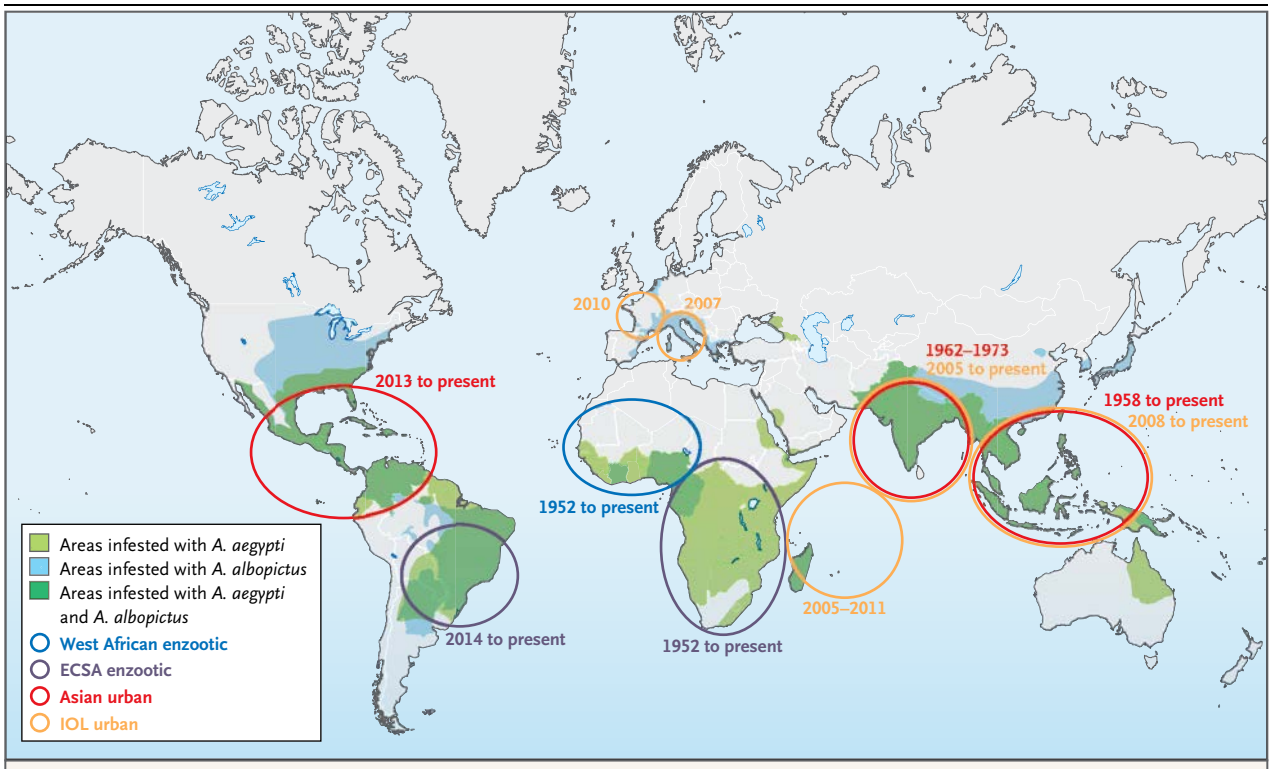

Figure 2. Origin, Spread, and Distribution of Chikungunya Virus and Its Vectors.

The map shows the African origins of enzootic chikungunya virus strains and the patterns of emergence and spread of the Asian lineage and Indian Ocean lineage (IOL) of the virus during epidemics since the 1950s, based on phylogenetic studies.[4,5] The distributions of the peridomestic vectors, *Aedes aegypti* and *A. albopictus*, are also shown. ECSA denotes eastern, central, and southern African.

EPIDEMIOLOGIC CHARACTERISTICS AND SPREAD

The initiation of urban chikungunya fever outbreaks follows spillover infection of humans from enzootic African transmission cycles; spillover infections have been documented in South Africa, Zimbabwe,[18] Cameroon,[19] Uganda,[20] and Senegal,[21] including small epidemics. Recent African outbreaks have also involved interhuman transmission by *A. albopictus*,[19,22] but evidence for the involvement of *A. aegypti* is mainly confined to Tanzania, Senegal, and Kenya.[23] The spread of the disease within Africa is not well understood, but after outbreaks reach the Indian Ocean basin and Asia, further dissemination by air travelers occurs frequently.[15,24] Urban chikungunya virus transmission patterns probably follow those observed for dengue virus, with social connections and routine movement of people among the homes of family and friends playing a key role in the spread of the virus by *A. aegypti*.[25] In locations where both *A. albopictus* and *A. aegypti* are present, the different abilities of the IOL and Asian strains to use these two urban mosquito vectors, which can be spatially segregated on the basis of their different preferred habitats,[26] result in different patterns of spread of these two chikungunya virus lineages.

CLINICAL SIGNS AND SYMPTOMS

Chikungunya fever is typically a rapid-onset febrile disease, characterized by intense asthenia, arthralgia, myalgia, headache, and rash. The abrupt onset of fever follows a mean incubation period of 3 days; when fever is present, the body temperature is usually higher than 39°C (Fig. 3).[27,28] In contrast to other arboviral diseases, such as dengue fever, the majority of persons who are infected have symptoms, with less than 15% of patients having asymptomatic seroconversion.[29] The onset of fever coincides with viremia, and the viral load can rapidly reach up to 10^9 viral genome copies per

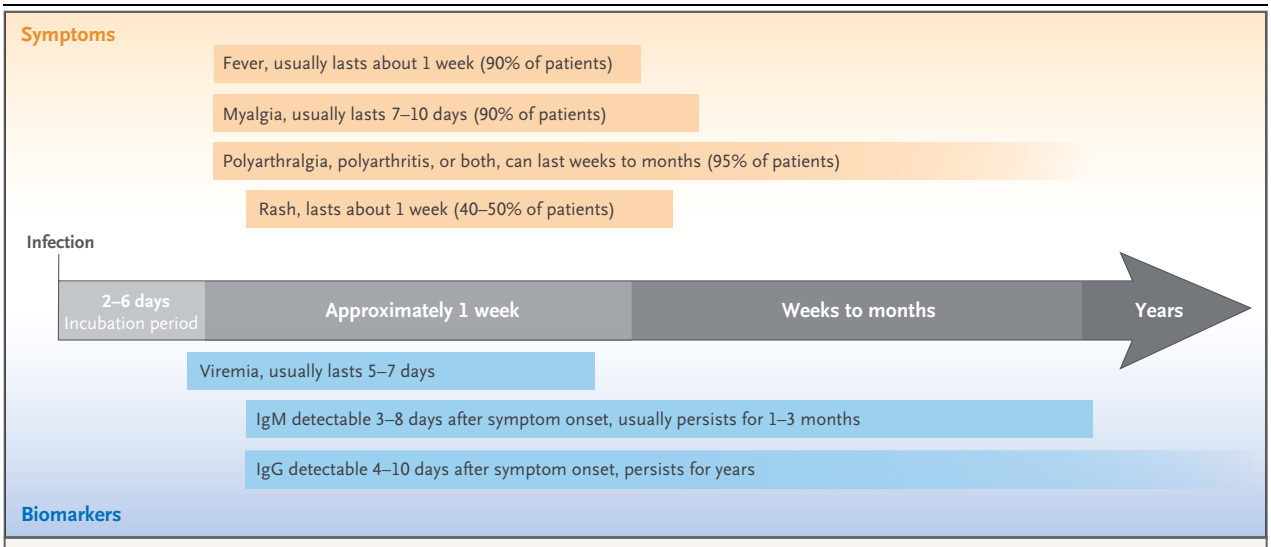

Figure 3. Timeline of Infection, Symptoms, and Biomarkers.

Shown is the chronology of viral replication in relation to the clinical and biologic signs of disease, including the biomarkers used in diagnostic assays to detect chikungunya virus infection (adapted from Suhrbier et al.[28]).

milliliter of blood. The intensity of the acute infection correlates with that of viremia, and the acute infection usually lasts 1 week, until viremia ends when IgM appears.[30,31] Soon after the onset of fever, severe myalgias and arthralgias occur; these are frequently so intense that patients have difficulty leaving the position they were in when their symptoms began. For differential diagnosis in regions where chikungunya virus circulates, the debilitating polyarthralgia has a positive predictive value greater than 80% for chikungunya virus viremia (see the interactive graphic, available at NEJM.org).[31,32] The joint pain is usually symmetric and localized in both the arms and legs (in 90% of patients); the large joints are almost invariably symptomatic, as are, to a lesser extent, the small joints and the vertebral column.[31] Periarticular edema and acute arthritis may also occur, in particular in the interphalangeal joints, wrists, and ankles, as well as pain along ligament insertions.

An interactive graphic is available at NEJM.org

Rash occurs in 20 to 80% of chikungunya fever cases, but it is also seen in other arboviral diseases, such as dengue fever. It is typically maculopapular and focused on the trunk, but it may also reach the face and involve the arms and legs, the soles, and the palms; it can be bullous in children. External ear redness is also observed, which may reflect chondritis and is evocative of chikungunya virus infection.[33] Less common, nonspecific signs and symptoms include lymphadenopathy, pruritus, and digestive abnormalities, which are more common after viremia has resolved. Feelings of faintness, fainting, confusion, and attention-deficit disorders are observed in the acute phase but may reflect the intensity of fever rather than chikungunya virus–specific pathogenesis. Rare complications can occur during the acute phase, including conjunctivitis, uveitis, iridocyclitis, and retinitis, which typically resolve.[33] These signs and symptoms have been described in geographic locations where no other arboviral disease outbreaks have been reported, which suggests that they were caused by chikungunya virus infection.

Patients with severe chikungunya fever requiring hospitalization tend to be older and to have coexisting conditions such as cardiovascular, neurologic, and respiratory disorders or diabetes, which are independent risk factors for severe disease.[33] Severe chikungunya fever can manifest as encephalopathy and encephalitis, myocarditis, hepatitis, and multiorgan failure. These rare forms can be fatal and typically arise in patients with underlying medical conditions. Hemorrhagic complications are rare and should lead to the consideration of alternative diagnoses, such as a coinfection with dengue virus or coexisting conditions such as chronic hepatopathy.

Neonates are another group at risk for severe infection associated with neurologic signs.

Whereas fetal infection appears to be extremely rare, the rate of infection of neonates born to viremic mothers and exposed to the virus during birth can reach 50%, leading to severe disease and encephalopathy in half and resulting in long-term neurologic sequelae.[34] Young children also tend to have severe disease. This age dependency of disease severity follows a U-shaped parabolic curve, with neonates and young children and the elderly at highest risk and with healthy adults usually having self-limited disease.

There is no licensed drug to limit chikungunya virus replication and improve clinical outcome, and only standard antipyretic and antalgic therapies are available for symptomatic treatment. Favipiravir,[35] as well as ribavirin plus interferon,[36] have been shown to have antiviral activity in vitro, but their safety and efficacy have yet to be demonstrated in clinical trials.

The major disease and economic burdens of chikungunya fever result not only from the high attack rate and severity of acute infection but also from chronic joint pain. This can be persistent or relapsing arthralgia that is located mostly in the distal joints, which may be associated with arthritis and may mimic rheumatoid arthritis (chronic inflammatory, erosive, and rarely deforming polyarthritis) in up to 50% of patients.[37] Chronic arthralgia can lead to persistent incapacitation requiring long-term treatment with nonsteroidal antiinflammatory and immunosuppressive drugs such as methotrexate, although their safety and efficacy also have yet to be demonstrated in clinical trials.[38]

DIAGNOSIS

The diagnosis of chikungunya fever is typically clinical, because the association of acute fever and arthralgia is highly predictive in areas where the disease is endemic and where epidemics have occurred.[31] The main laboratory finding is lymphopenia, which, when the lymphocyte count is less than 1000 per cubic millimeter, is closely associated with viremia. Other laboratory abnormalities include thrombocytopenia, increased levels of aspartate aminotransferase and alanine aminotransferase in blood, and hypocalcemia. A definitive diagnosis relies on virus detection through reverse-transcriptase–polymerase-chain-reaction (RT-PCR) testing during the viremic phase (the first week). RT-PCR can be designed in a multiplex format to simultaneously detect several other arboviruses, such as dengue virus, which can be very useful for triage of patients. Chikungunya virus culture in a variety of cells permits further virologic characterization but has no added value over RT-PCR in clinical practice and is not performed routinely. Serodiagnosis is facilitated by the limited antigenic diversity of chikungunya virus and extensive cross-reactivity of the antibodies induced by different strains.[5] Serum IgM is detectable from day 5 (and even earlier) to several months after the onset of illness and is also considered diagnostic. Seroconversion can also be detected as an increase in IgG by a factor or 4 or more between acute-phase and convalescent-phase serum samples. There is no specific assay for assessing chronic signs and symptoms associated with chikungunya fever, although elevated levels of C-reactive protein and proinflammatory cytokines correlate with disease activity, as do anti–chikungunya virus IgG levels and persistent anti–chikungunya virus IgM.[37] Persistence of high antibody titers and their correlation with chronic disease may indicate delayed antigen clearance rather than viral persistence.

PATHOPHYSIOLOGICAL CHARACTERISTICS

Chikungunya virus can easily be cultivated in a wide variety of cell lines of insect and mammalian origin.[28,39] In vivo cell tropism has been investigated in rodent and nonhuman primate models, as well as in human tissue samples. In immunocompetent mice, chikungunya virus targets fibroblasts in the dermis around the injection site and is rapidly controlled by type I interferon responses.[40-43] In neonatal mice and mice partially or completely deficient in type I interferon signaling, chikungunya virus disseminates systemically, leading to viremia and a burst of viral replication in the liver and to intense replication in muscle, joint, and skin fibroblasts (see the interactive graphic at NEJM.org).[39] This tropism seems to mirror that observed in biopsy samples from humans, although a detailed analysis of chikungunya virus–infected human tissues has not been performed. In contrast to other acute viral infections, in the acute phase of chikungunya virus infection, the sites where symptoms focus are typically infected, especially skeletal muscles, myotendinous insertions, and joint capsules.[42]

In animal models, chikungunya virus also disseminates to the central nervous system (CNS): it infects choroid plexuses, reaches the cerebrospinal fluid, and infects the meningeal and ependymal cells that envelop the CNS.[42] Chikungunya virus is not known to target brain microvessel endothelial cells or to infect neurons. However, infection of the meninges and ependymal cells, as well as the resulting cytopathic effects and the host responses they trigger, may affect underlying neuronal cells, and this may account for the CNS signs and symptoms associated with severe chikungunya fever. Experimental infection of pregnant animals and investigation of human placentas from viremic mothers have shown that, in contrast to other alphaviruses, chikungunya virus does not directly infect trophoblastic cells but is probably transmitted to neonates through maternal–fetal blood exchange during delivery.[42]

The contribution of chikungunya virus infection of myeloid cells to the pathogenesis of acute and chronic chikungunya fever remains incompletely understood.[28,39] Whereas myeloid cells do not seem to contribute substantially to viral replication at the early stage of infection, interactions of chikungunya virus with monocytes and macrophages may play an important role in the inflammatory responses during the acute and chronic phases of disease; although the control of chikungunya virus replication critically requires type I interferon sensing by nonmyeloid cells, myeloid cells are probably involved in the clearance of infected cell debris, which may trigger proinflammatory responses related to chronic joint pain. The determination of whether persistent chikungunya virus replication, lack of virus antigen clearance, or both contribute to chronic arthralgic symptoms requires further studies with animal models and human samples.

CONTROL OF THE SYMPTOMS AND SPREAD OF DISEASE

Other than antiinflammatory drugs to control symptoms and joint swelling, there are no specific therapeutic agents to treat infected persons and no licensed vaccines to prevent chikungunya fever. In animal models, passive immunotherapy has been shown to be efficacious in the prevention and cure of chikungunya virus infection,[44] but this approach has yet to be tested in humans; it will be particularly important to test this approach in neonates born to viremic mothers (see, e.g., ClinicalTrials.gov number NCT02230163).[34]

Until there is a treatment or vaccine, the control of chikungunya fever, like that of dengue fever, will rely on vector reduction and on limiting the contact between humans and the *A. aegypti* and *A. albopictus* mosquitoes.[45] These efforts generally focus on reducing or treating standing water and containers for water storage, including backyard, nondegradable trash containers where eggs are laid and larvae develop. Reducing the populations of these mosquitoes through traditional larvicide and adulticide applications has had limited success in controlling dengue fever, particularly when treatments are not designed to penetrate the houses where many adult female mosquitoes rest and feed (male mosquitoes do not bite and thus do not transmit chikungunya virus). Novel strategies for vector control include the release of transgenic *A. aegypti* engineered to carry a late-acting lethal genetic system.[46] Another promising approach to reducing transmission is the use of wolbachia bacteria, which, when introduced into *A. aegypti* or *A. albopictus* mosquitoes, reduce their vector competence for chikungunya virus and dengue virus.[47-49] Ways of limiting contact between infected mosquitoes and people include wearing protective clothing, sometimes impregnated with insecticides, or wearing repellents. Insecticide-impregnated curtains can limit the entry of endophagic mosquito vectors into homes to reduce dengue fever,[50] but insecticide resistance poses a challenge to this approach and other control efforts. Education and control in regions without a history of dengue fever should focus on the daytime biting behavior of *A. aegypti* and *A. albopictus* mosquitoes and their tendency to enter houses.

THE FUTURE OF CHIKUNGUNYA AND RESEARCH PRIORITIES

BASIC RESEARCH

Although key advances have been made in understanding the biologic aspects and pathogenesis of chikungunya fever, many questions critical to the development of targeted therapeutic and preventive strategies remain unanswered. The high-resolution crystal structure of the chikungunya virus envelope glycoprotein complexes has been determined,[2] but the host-cell receptor or receptors and the molecular mechanisms of the entry

of the virus into human and mosquito cells remain unknown. Although model alphaviruses such as Sindbis virus and Semliki Forest virus have been intensively studied for decades, the specifics of chikungunya virus replication and the host-cell response remain poorly understood. Deciphering the basic mechanisms of chikungunya virus replication with the use of traditional cell and molecular virologic approaches, as well as with high-throughput small interfering RNA and small-compound library screening, will be key for the development of antiviral agents. The innate and adaptive immune responses to acute chikungunya virus infection have received much attention,[37,40,51] yet the pathogenesis of chronic arthralgia and the basis for the variation in long-term outcome among patients remain poorly characterized. These issues will require large and systematic patient cohort studies, compilation of detailed clinical data, analyses of blood and tissue samples, the discovery of biomarkers related to disease severity in acute versus chronic disease, and genome studies involving patients.

Although progress has also been made to understand the basic mechanisms of chikungunya virus evolution and outbreak emergence, additional work is needed to elucidate the molecular mechanisms of adaptation of the virus to mosquito vectors, which might lead to new targets for control strategies. We are just beginning to understand the adaptive landscape (i.e., the fitness for infection and transmission of a wide range of viral mutants) of chikungunya virus and other arboviruses at a superficial level.[9,52] Improved knowledge of mutational processes and of protein structure and function is needed to improve predictions regarding the emergence of chikungunya virus and other zoonotic arboviruses through host range changes. The identification of chikungunya virus receptors in the midgut of mosquitoes and the determination of entry mechanisms are needed to better understand vector specificity.

PREVENTION AND CONTROL

Unfortunately, the immediate prospects for the control of chikungunya fever are poor, as indicated by the lack of success with dengue fever for many decades. Furthermore, the rapid development of insecticide resistance in mosquitoes threatens the limited vector-control strategies that are available in some regions. Education of the public regarding the reduction of sources of standing water that serve as larval habitats for *A. aegypti* and *A. albopictus*, combined with efforts to kill adult female mosquitoes within and around houses and to limit the exposure of humans to these mosquitoes, remain the main strategies for control of chikungunya fever until new approaches like those discussed above can be developed.

Chikungunya fever represents a simpler vaccine target than dengue fever, because it has much more limited antigenic diversity and no evidence of immune enhancement of disease. Several promising chikungunya fever vaccine candidates have reached late preclinical or phase 1 clinical testing,[8,53] but final development will require major commercial investments. The licensure of vaccines and therapeutics will be challenging because of the difficulty in identifying locations of predictable chikungunya fever incidence or emergence to conduct affordable efficacy trials, as well as the difficulty in predicting future markets. The identification of sites for clinical efficacy trials will be a major financial and logistic challenge, because chikungunya fever surveillance usually wanes after epidemics peak, and estimates of residual endemic incidence are needed to predict the scopes and costs of trials, as well future markets. Thus, improved chikungunya fever surveillance involving affordable, point-of-care diagnostics will be critical to many aspects of chikungunya fever prevention and control and remain top research priorities. Distinguishing chikungunya virus infection from dengue virus infection is especially critical because only the latter can lead to life-threatening hemorrhagic fever, which requires hospitalization of the patient and careful management of the patient's condition.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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