# Chikungunya infection: de-linking replication from symptomatology reveals the central role of muscle

**Anne Moscona**

Department of Pediatrics, Microbiology & Immunology, and Department of Physiology & Cellular Biophysics, Columbia University College of Physicians & Surgeons, New York, New York, USA.

**Chikungunya virus (CHIKV) is an emerging arbovirus, endemic in many parts of the world, that is spread by travelers and adapts to new mosquito vectors that live in temperate climates. CHIKV replicates in many host tissues and initially causes a self-limiting febrile illness similar to dengue. However, in 30%–40% of cases, CHIKV also causes long-term painful and debilitating muscle and joint pain, the pathogenesis of which remains unknown. In this issue of the *JCI*, Lentscher et al. engineered a skeletal muscle–restricted CHIKV to show that while musculoskeletal disease requires viral replication in affected muscle, muscular pathology is mediated by host immunological factors. These findings de-link viral replication and disease symptoms, illuminate the virus-host interplay in CHIKV symptomatology, and raise the possibility that immune modulation is a therapeutic option. The results also highlight possible solutions to existing vaccine barriers and provide insights that may apply to other viral diseases.**

## Chikungunya is a uniquely dangerous emerging virus

Chikungunya virus (CHIKV) is a member of the Old World *Alphavirus* genus of the *Togaviridae* family. It is an arbovirus that causes widespread disease in the developing world and an increasing number of outbreaks in temperate climates. The virus is small (70 nm) and enveloped, carrying a single-stranded, message-sense RNA genome of about 11,800 nucleotides. Several features set CHIKV apart from related mosquito-borne febrile disease–causing viruses, making its emergence a major global concern. Its high rates of infection and alternating rural and urban transmission cycles mean that it spreads quickly and across broad geographic areas. While several emerging viruses are expanding in range as a result of climate and socioenvironmental changes that affect transmission, CHIKV expansion is aided by the capacity of the virus to adapt to additional mosquito vectors that live in temperate climates (1–4), the high burden of disease caused by large urban outbreaks, and the fact that infected humans can seed transmission in naive areas, setting up transmission and new outbreaks (5–7). CHIKV is rapidly establishing a stronghold in a widening geographic range, at a time when there are no effective antivirals or available vaccines and a lack of understanding of the basis for the severe long-term sequelae of infection. Moreover, while CHIKV infection initially causes a self-limiting febrile illness similar to that of dengue, in 30%–40% of cases it leads to a persistent, painful, and debilitating rheumatic disease (8). The pathogenesis of these long-term musculoskeletal symptoms after CHIKV infection is not understood.

## Three strides toward pathogenesis, and a potential therapy

In this issue of the *JCI*, Lentscher et al. clearly define a role for CHIKV replication in skeletal muscle relating to the inflammation that is the hallmark of CHIKV disease (9). Along the way, the paper makes three important distinct but interconnected contributions. First, the study advances a creative experimental strategy that takes advantage of the cell-type specificity of the host microRNA-mediated (miRNA-mediated) RNA silencing process in order to study pathogenesis; the authors engineered a virus that has diminished replication in muscles. This method was used to determine whether the site of replication mediated symptomatology. Second, the authors convincingly established that indeed CHIKV muscle disease is tissue specific, requiring virus replication in the affected muscle tissue, despite the capacity of the virus to replicate in many other tissues. Third, the experiments cleanly de-linked viral replication from symptoms of disease, documenting definitively that the disease manifestations are a function of a host immune response and can be mitigated by ablating that specific response. The authors identified IL-6 as an important mediator of inflammation in this setting, opening the door to therapeutic strategies (9).

## Co-opting a host mechanism to dissect tissue specificity

The Lentscher et al. study takes advantage of the host RNA silencing machinery, thereby diminishing viral replication in specific tissues while permitting viral replication elsewhere (9). This RNA silencing method may be a broadly useful approach to help understand pathogenesis and dissect the basis for tissue-specific disease manifestations. miRNAs are small noncoding RNAs that contribute to posttranscriptional regulation of protein expression (10). Crucially for this paper's experimental strategy, miRNAs are often expressed in a tightly cell type–specific fashion (11). The authors engineered a CHIKV strain with sequences complementary to a tissue-specific miRNA in order to restrict viral replication only in cells expressing

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that particular restrictive miRNA—in this case, the skeletal muscle. The researchers incorporated sequences complementary to skeletal muscle–specific miRNA miR-206 into the CHIKV genome, generating a skeletal muscle–restricted CHIKV strain (SKE) along with a mismatch sequence control CHIKV strain (SKE MM). These viruses proved to be perfect tools. SKE was specifically restricted by miR206 in culture and restricted in replication in only the skeletal muscle in mice (while replicating normally at other sites); on the other hand, SKE MM showed neither restriction. Armed with these tools, the authors examined the specific impact of infection at this key site. This is an approach that could be applied, as the authors note, to other sites of CHIKV replication, but also to a range of other viral illnesses for which the precise sites of replication associated with disease are enigmatic (9).

## Replication in muscle is necessary for muscle disease

CHIKV spotlights a puzzle for a broad swath of viral diseases; the virus infects a variety of cell types and replicates at many sites within the host, but disease is tissue specific. The general concept of a requisite interplay between host and pathogen in pathogenesis is often mentioned in the literature but only rarely elucidated. The two viruses differing only in being muscle restricted (SKE) or permissive (SKE MM) were similar in their induction of viral load and tissue burden of infection in mice in every way but one: musculoskeletal disease (muscle inflammation) occurred only with SKE MM. Replication in muscle is required for disease, but it is not enough to elicit pathology (9).

## Necessary but not sufficient: host inflammation mediates muscle disease

The mice infected with SKE virus (restricted in skeletal muscle cells) had less inflammation, T cell infiltration into the interosseous muscle (the site of muscle disease in this model), and inflammatory mediators at the site, but only in the local skeletal muscle. SKE MM infection recruited the inflammatory mediators in the muscle seen in humans with CHIKV, including IL-6 (9). However, the global inflammatory picture in the mice outside the interosseous muscle was no different after infection with the two viruses. Pathogenesis was truly local. The authors were then perfectly positioned to ask: is disease due to viral replication or due to the influx of inflammatory mediators? Remarkably, blocking IL-6 with receptor blocking antibody ablated disease in the muscle. Even in the face of rampant SKE MM viral replication in the muscle, without IL-6 there was limited tissue disease. As the authors note, the question solved here for CHIKV also applies to other viruses that induce myositis, including influenza, where the relative contributions of viral replication and the host immunologic response to the tissue damage are unknown. The tissue restriction strategies used here may be applicable to other tissue-specific diseases as well (9). Moreover, the finding that IL-6 is key to disease induction and the possibility that an existing anti–IL-6 antibody (12) might ameliorate disease in humans is an exciting example of therapy based on disease pathogenesis, lightening the standard focus on targeting solely viral factors for therapies.

## CHIKV vaccines: a valuable attenuation strategy?

While a CHIKV vaccine should be a public health priority, its development has to overcome the difficulties associated with multiple strains, unpredictable outbreaks, and vaccine-induced symptoms (13, 14). The findings in Lentscher et al. may provide a solution to precisely this latter problem (9). A significant question that emerges from this work is whether a vaccine from engineered SKE virus, or a derivative of it, would protect. Although there are no licensed therapies or vaccines to date, over the last ten years progress has been made toward several vaccine candidates (15). Live vaccines would be ideal in terms of effective and long-lasting cross–CHIKV clade protection, but they would face the challenge of achieving the right balance between viral attenuation (dampening of viral replication) and immunity induction (eliciting a protective response). Attenuation must achieve safety yet generate enough antigen to elicit protective immunity. The first promising vaccine candidate, a live attenuated strain generated by tissue culture passage (16), was highly immunogenic in phase II trials, but was derailed because it caused transient musculoskeletal symptoms in 8% of vaccinees (17) and the attenuation was unstable, with frequent reversion at the attenuating sites (18, 19). Newer attenuated CHIKV vaccine candidates include one bearing an internal ribosome entry site (IRES) replacing the CHIKV subgenomic promoter, preventing replication in the mosquito vector and attenuating replication in humans while protecting nonhuman primates against challenge (20, 21). Other candidate strategies under hopeful consideration include a nonreplicating virus-like particle assembled from engineered CHIKV proteins (22, 23), a recombinant live-attenuated measles virus vectored vaccine expressing CHIKV structural proteins (24), and a recombinant Eilat/CHIKV chimeric virus that is replication defective in mosquitoes and protective in nonhuman primates (25). The property conferred by the miRNA complementary sequences in the SKE CHIKV strain described by Lentscher et al.—restriction of replication in skeletal muscle—could eliminate the concern that live virus vaccines may target skeletal muscle and produce disease (9). These viruses will replicate enough to induce immunity without risking the hallmark disease manifestations. Replication, de-linked from disease symptoms, could then be modulated by additional attenuating mutations, as desired, to achieve an optimal level of immune response. Beyond CHIKV, it is tempting to consider whether engaging the miRNA system to restrict viral replication in specific target tissues may be a broader attenuation strategy for viral vaccine candidates.

Address correspondence to: Anne Moscona, 701 West 168th Street, New York, New York, 10032, USA. Phone: 347.366.2459; Email: am939@cumc.columbia.edu.

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