Chikungunya disease is a severely debilitating, mosquito-borne, viral illness that has reached epidemic proportions in Africa, Asia, and the islands of the Indian Ocean. A mutation enhancing the ability of the chikungunya virus (CHIKV) to infect and be transmitted by Aedes albopictus has increased the geographical range at risk for infection due to the continuing global spread of this mosquito. Research into disease pathogenesis, vaccine development, and therapeutic design has been hindered by the lack of appropriate animal models of this disease. The meticulous study reported in this issue of the JCI by Labadie et al. is one of the first reports describing CHIKV infection of adult immunocompetent nonhuman primates. Using traditional and modern molecular and immunological approaches, the authors demonstrate that macaques infected with CHIKV are a good model of human CHIKV infection and also show that persistent arthralgia in humans may be caused by persistent CHIKV infection of macrophages.

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A nonhuman primate model of chikungunya disease

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associated, but no data support the suggestion (18). Global travel and commercialization are likely contributors through rapid movement of infected humans and vector invasions, respectively.

Mouse models of CHIKV and RRV

As with dengue fever, the lack of a good animal model replicating human chikungunya fever symptoms and pathology has been a major obstacle for understanding the course of infection, spectrum of disease severity, and persistence of symptoms. A mouse model for RRV, predating the recent work with CHIKV, incorporates both traditional and state-of-the-art molecular and immunological approaches to evaluate a nonhuman primate model for this important, emerging, vector-borne disease—specifically, infection and observed symptoms in a laboratory-infected nonhuman primate model of chikungunya disease. In their study in this issue of the *JCI*, Labadie et al. describe the stages of symptoms of CHIKV infection seen in their primate model as having 3 phases: acute, subacute, and chronic (1). These symptoms may be correlated with recently published observations of the symptoms in human cases of chikungunya fever (4, 5, 12, 23). In the acute phase, nonhuman primates show high viremia (approximately 10⁸–10⁹ viral RNA copies/ml), fever, and rash. They also have increased levels of aminotransferase, including aspartate transaminase (AST) and alanine transaminase (ALT), as well as increased expression of IFN, MCP-1, and IL-6. The nonhuman primates also have abnormal blood chemistries, including lymphopenia, monocytopenia, and granulocytosis. Humans in the acute phase of chikungunya fever present with fever, fatigue, headache, myalgia, and rash as well as increased AST and ALT. Viremia in humans ranges from 10⁷ to 10⁹ viral RNA copies/ml. Leukopenia and thrombocytopenia are common. During the subacute phase in nonhuman primates, there is macrophage infiltration, and CHIKV is present in lymphoid tissue, liver, meninges, joints, and muscle. The subacute phase is not well characterized in humans, but includes the persistence of myalgia, arthralgia, and tenosynovitis. In the chronic phase in nonhuman primates, there was CHIKV persistence in macrophages, lymphoid tissue, and liver tissue up to 2 months after infection. The chronic phase in humans includes persistent and recurrent arthralgia that can last for months to years.

Primate models of CHIKV

The current study reported by Labadie et al. incorporates both traditional and state-of-the-art molecular and immunological approaches to evaluate a nonhuman primate model for this important, emerging, vector-borne disease—specifically, infection of long-tailed macaques (*Macaca fascicularis*) with a CHIKV isolate from a patient infected during the recent La Réunion outbreak (1). The symptoms and pathology observed in the nonhuman primate model resembled many of those previously reported for CHIKV infection in humans, and so the data are a significant contribution to our understanding of the disease (Figure 1 and refs. 5, 23). It is gratifying that the study was not driven by the use of highly sensitive molecular techniques that do not distinguish between infectious and defective virus, but incorporated more traditional infectivity and immunological assays that can be readily interpreted by a broad audience in the context of previous work. The synergism of using multiple techniques and the duration of the study has begun to explain the mechanisms underlying the long-term persistence of chikungunya fever symptoms. The reported correlation between dose and disease severity (more severe at a higher dose) is interesting and may explain, at least in part, the range of symptoms observed in humans. The doses used by the authors are within ranges likely delivered by mosquitoes (24). It would have been interesting if some primates had been challenged by CHIKV-infected mosquitoes, given the increasing evidence that chikungunya fever has an immunological component. Mosquito saliva is known to have immunomodulatory activity, and, for several viruses (for example, West Nile virus), virus delivery in the context of saliva can influence infection establishment, dissemination, and severity (25, 26). As Labadie et al. state, the

<table>
<thead>
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<th>Figure 1</th>
<th>Comparison of symptoms resulting from naturally acquired human CHIKV infection and observed symptoms in a laboratory-infected nonhuman primate model of chikungunya disease. In their study in this issue of the <em>JCI</em>, Labadie et al. describe the stages of symptoms of CHIKV infection seen in their primate model as having 3 phases: acute, subacute, and chronic (1). These symptoms may be correlated with recently published observations of the symptoms in human cases of chikungunya fever (4, 5, 12, 23). In the acute phase, nonhuman primates show high viremia (approximately 10⁸–10⁹ viral RNA copies/ml), fever, and rash. They also have increased levels of aminotransferase, including aspartate transaminase (AST) and alanine transaminase (ALT), as well as increased expression of IFN, MCP-1, and IL-6. The nonhuman primates also have abnormal blood chemistries, including lymphopenia, monocytopenia, and granulocytosis. Humans in the acute phase of chikungunya fever present with fever, fatigue, headache, myalgia, and rash as well as increased AST and ALT. Viremia in humans ranges from 10⁷ to 10⁹ viral RNA copies/ml. Leukopenia and thrombocytopenia are common. During the subacute phase in nonhuman primates, there is macrophage infiltration, and CHIKV is present in lymphoid tissue, liver, meninges, joints, and muscle. The subacute phase is not well characterized in humans, but includes the persistence of myalgia, arthralgia, and tenosynovitis. In the chronic phase in nonhuman primates, there was CHIKV persistence in macrophages, lymphoid tissue, and liver tissue up to 2 months after infection. The chronic phase in humans includes persistent and recurrent arthralgia that can last for months to years.</th>
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<tr>
<td><strong>Acute phase</strong></td>
<td><strong>Subacute phase</strong></td>
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<tr>
<td>Fever, fatigue, headache, myalgia, rash</td>
<td>Fever, rash</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>Lymphopenia</td>
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<td>Thrombocytopenia</td>
<td>Monocytopenia</td>
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<td>Increased AST, ALT</td>
<td>Granulocytosis</td>
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<td>Viremia with CHIKV in multiple tissues</td>
<td>Increased AST, ALT</td>
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<td>Increased IFN, MCP-1, IL-6</td>
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<td>Viremia with CHIKV in multiple tissues</td>
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**Chronic phase**

| Persistent and recurrent arthralgia | CHIKV persistence in macrophages, lymphoid organs, liver |
| Muscle inflammation | |
macaque model should help in the development of new therapeutic or prophylactic strategies against chikungunya fever and facilitate testing of new interventions (1). This work represents a considerable advance over early primate studies (27, 28), and development of a nonhuman primate model of CHIKV infection has been a focus of several groups. The in utero infections observed during the La Réunion epidemic have been evaluated in a macaque model (29). Importantly, a macaque model has been developed at the NIH laboratories to determine the efficacy of a much-needed CHIKV vaccine and has demonstrated excellent protection against challenge with virulent CHIKV (30). This model, like that reported here by Labadie et al., mimicked key features of human chikungunya disease.

Labadie et al.’s current work in macaques (1) shows a good correlation with previously published work with mice. With little neurological involvement and virus prevalence in immune tissues and muscles, the present model shows promise. The authors admit that a limitation of their model is the lack of joint and muscle pathology in the macaques, but note that the occurrence of these symptoms in humans is restricted to the most severe cases, although recent reports have stated that arthralgia/arthritis actually affects 73%–80% of patients (5). Another limitation of the Labadie et al. paper is that no serology studies were done. It would be interesting to see the level and types of antibodies that the macaques produced in response to CHIKV infection.

**Immunopathology during CHIKV infection**

Labadie et al. present a compelling argument for the importance of macrophages in causing CHIKV-induced disease (1). Macrophages have been shown to be one of the keys to the destructive muscle necrosis, and possibly viral persistence, seen in RRV infection in mice. These early observations of RRV infection in mice are substantiated in the work by Labadie et al. Rulli et al. discussed the recent RRV data showing that macrophages are a key mediator of muscle necrosis and pathology and that, in vitro, RRV can persistently infect macrophages and upregulate release of IFN-γ, TNF-γ, and monocyte chemoattractant protein–1 (MCP-1; MCP-1; ref. 31). Macrophage infiltration into muscles and joints in RRV-infected mice was responsible for the production of cytokines and chemokines, such as TNF-α, macrophage inflammatory protein–1α (MIP-1α), MCP-1, IL-1β, and IFN-γ (19). In the CHIKV study by Labadie et al., infected macaques showed increased expression of IFN-α/β, CCL2, CCL3, CCL4, IL-6, and IFN-γ and moderate increases of TNF-α (1). These findings are what would be expected of a viral infection inducing a strong macrophage response. The typical antiviral response in humans is characterized by the induction of cytokines such as IFN-α/β, IFN-γ, IL-12, and IL-18 and the activation of macrophages, NK cells, dendritic cells, neutrophils, and complement. TNF-1, IL-1, and IL-6 are produced by immune cells as signals of inflammation. Cytokines IL-4 and IL-10, which are not upregulated during viral infection, were not upregulated in the current study (1), again emphasizing the importance of macrophages.

**Wild primates and CHIKV transmission cycles**

The demonstration of *M. fascicularis* susceptibility to CHIKV infection (1) raises an interesting epidemiological question that merits further investigation. Within what can be considered as the endemic/original geographical region of Africa in which CHIKV circulates, primates have been implicated in a transmission cycle that does not involve humans. McIntosh et al. (32) reported CHIKV seropositive, wild-ranging vervets (*Cercopithecus aethiops pygerythrus*) and baboons (*Papio ursinus*) collected in the 1962 endemic area of southern Rhodesia and experimentally recorded that viremias in these animals lasted for 3–4 days in both species, “at levels adequate to infect significant numbers of susceptible mosquitoes” (33). In Asia, the potential involvement of primates in the transmission cycle is unclear. Inoue et al. reported an IgM seropositive rate of 14.8% for *M. fascicularis* in the Philippines (34), and Marchette et al. reported seropositive monkeys in Malaya – although 15 of 16 were also positive for other related viruses, which can compromise the tests’ accuracy (35). However, Peiris et al. found no CHIKV positivity among 115 wild toque macaques (*Macaca sinica*) from Sri Lanka (36). The suggestion that there is a sylvatic cycle in Asia must be treated with caution, since evidence of infection in a wild animal must not be interpreted as proof of involvement in a transmission cycle. Specific criteria must be met when considering whether a vertebrate is involved in virus transmission. Basically, the vertebrate must be spatially and temporally coincident with a known vector, be naturally fed upon by that vector, and produce viremias of sufficient magnitude and duration to infect the vector. Finally, transmission between the vertebrate and vector must be experimentally demonstrated. To date, these criteria have not been satisfied for primates in Asia, so we cannot assume that primates play any significant role in the Asian CHIKV transmission cycle. This is a gap in our knowledge that needs to be filled, and one to which the study by Labadie et al. contributes (1). *M. fascicularis* is widespread throughout Asia (37) and has been introduced into Hong Kong, Indonesia, Mauritius, and Palau. It is interesting that the species is present in large numbers on Mauritius (30,000 estimated; ref. 38), but there are no compelling data to implicate its involvement in the large chikungunya fever epidemic in the region (C. Chastel, personal communication).

Overall, the development and characterization of primate models for chikungunya fever represent a significant advance in the field that has already increased our understanding of the disease and facilitated evaluation of new potential vaccines. It is hoped that identification of the underlying mechanisms of the disease will be translated into a reduction of the consequences of infection and new preventive strategies that ultimately reduce disease incidence.

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While erudite cell biologists have for many decades described singular immotile appendages known as primary cilia to be present on most cells in our bodies, ciliary function(s) long remained an enigma. Driven largely by an ever increasing number of discoveries of genetic defects in primary cilia during the past decade, cilia were catapulted from a long lasting existence in our bodies, cilial function(s) long remained an enigma. Driven largely by cilia-related cystogenic diseases.

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Based on a flood of recent evidence, primary cilia are now heralded as sensory organelles for detection and transmission of a broad range of cues from the extracellular environment of cells (1), including mechanical and chemical information as diverse as fluid flow in kidneys, mechanical bone deformation, and light and odorant detection (2). By processing such physical and chemical information from the environment into molecular signals in development and postnatal growth and homeostasis, cilia can affect cell differentiation and polarity and cell cycle control (3).

Genetic damage to primary cilia results in a spectrum of perplexing disorders with seemingly disparate manifestations, now classified as ciliopathies. A growing list of bona fide ciliopathies at present includes Bardet-Biedl syndrome (BBS), nephropathies (NPHP), and Senior-Loken syndrome (SNLS), just to name a few. Common