Recent developments and challenges in mouse models of Chikungunya virus infection

“...*in vivo* studies have provided us with invaluable information to help understand ... Chikungunya virus infections and enable the testing of novel therapeutic interventions.”

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The re-emergence of Chikungunya virus (CHIKV), an *Alphavirus* that causes debilitating fever, nausea, headache and polyarthralgia in humans, is responsible for major disease outbreaks in many regions and rekindled interest in studying the pathophysiology of CHIKV infection. There is currently no effective antiviral or vaccine against this disease. Mouse models of CHIKV infection have been developed to investigate CHIKV pathogenesis; however, each model presents both advantages and disadvantages. Nevertheless, *in vivo* studies have provided us with invaluable information to help understand the mechanism of CHIKV infections and enable the testing of novel therapeutic interventions.

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CHIKV is a positive-sense ssRNA virus, belonging to the family of *Togaviridae*, genus *Alphavirus*. It was first isolated in Tanzania, Africa in 1952 [1] and mainly transmitted by *Aedes* mosquitoes, *Aedes aegypti* and *Aedes albopictus* [2,3]. CHIKV can cause diseases in human characterized by fever, nausea, rash, headache, myalgia and acute/persistent polyarthralgia [4,5]. In rare cases, neurological complications can be observed in neonates as well as in adults [6,7]. In recent years, the re-emergence of CHIKV has led to major outbreaks in many regions [8,9], which can result in increasing social and economic

burdens. Currently, there are no known vaccines or specific treatment regimens available for CHIKV infection [10].

Ideally, to obtain a more precise understanding of host–virus interaction and disease pathophysiology, clinical research studies in CHIKV-infected patients are recommended. However, research studies in humans are hampered by ethical issues and difficulties in acquiring human tissues for downstream investigation. To overcome this limitation, mouse models of CHIKV infection developed to gain further insights into CHIKV disease pathogenesis and evaluate potential novel antiviral interventions. A major advantage of using mouse models is the ability to obtain tissue samples at various time-points postinfection (pi) from different mice of the same genetic background to better understand the pathogenesis of CHIKV.

Mice of different genetic backgrounds and ages were used by various research groups to generate mouse models of CHIKV infection. Most of the models exhibited CHIKV tropism in muscles and joints similar to those observed in humans. A study by Couderc *et al.* demonstrated that 6-day-old and 9-day-old wild-type (WT) C57BL/6J mice, when infected intradermally (id.) in the ventral thorax, showed muscle weakness leading to paralysis from day 6 pi. Only 50% of the 9-day-old infected mice survived, while all 6-day-old infected mice died by day 12 pi. From day 3 pi, low levels of infectious CHIKV were found in the blood, spleen, liver and brain, and higher levels of virus were observed in the skin, joints and muscle [11]. A second investigation by the same group infected 9-day-old WT C57BL/6J mice subcutaneously (sc.) in the flank region, and showed close to 60% mortality rate from day 10

Keywords

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pi with viremia being observed in blood, muscle, liver, lung and brain as early as day 1 pi [12]. Similarly, 2–3-day-old outbred mice, CD-1 and ICR mice, infected sc. with CHIKV, displayed disease symptoms such as lethargy, weight loss, dragging of hind limbs and hair loss around the site of inoculation from day 7 pi [13]. However, these symptoms were not recapitulated in adult WT mice [11,12].

“The successful replication of disease progression in the humanized mouse model will enable the evaluation of clinical Chikungunya virus isolates with distinct host response profiles, disease outcomes or viral load properties.”

Since adult WT mice were resistant to CHIKV infection via id. and sc. routes of inoculation, immunocompromised mouse models such as adult IFN- α / β R knockout mice were used to develop an *in vivo* adult model of CHIKV infection. It was reported in earlier studies that endogenous IFN play a critical role in controlling CHIKV infection in humans and mice. Therefore, it was hypothesized that IFN- α / β R knockout mice would have increased susceptibility to CHIKV. As expected, adult IFN- α / β R-knockout mice infected via id. in the thorax manifested CHIKV-associated symptoms such as lethargy, muscle weakness of the limbs and 100% fatality at day 3 pi. These mice also exhibited viremia and histological changes in muscle, liver, joints, spleen, skin and brain [11]. Although these investigations did not accurately decipher CHIKV pathogenesis since these mice were either immunologically naive or impaired, they provided useful information on the *in vivo* CHIKV tropism.

Interestingly, when the virus inoculation site was changed to the ventral side of each hind foot in adult 6-week-old female WT C57BL6/J mice, typical disease outcomes including foot swelling from day 6 pi, and increased inflammatory cell infiltrates comprising monocytes, macrophages and natural killer (NK) cells were present in the foot, joint, synovial membranes, tendon connective tissues, lymph nodes, spleen and muscle. CHIKV infection also resulted in elevated virus titers in feet, muscle, liver, spleen and lymph nodes and increased levels of proinflammatory mediators such as IFN- γ , IFN- α / β , TNF- α , IL-6 and MCP-1 in the mouse model [14]. Likewise, a separate study by Morrison *et al.* showed that 2-week-old WT

C57BL6/J mice, when inoculated in the left rear footpad exhibited swelling and edema of the left foot at day 2 pi and inflammatory changes of musculoskeletal tissues leading to arthritis, tenosynovitis and myositis [15]. However, this CHIKV-induced swelling was confined to the inoculated foot, suggesting inefficient viral dissemination in the mouse [14,15]. Two other studies in which CHIKV was inoculated intranasally in adult Balb/c and NIH Swiss mice exhibited weight loss and CHIKV-related pathologies such as severe spongiform changes, formation of apoptotic bodies and microglial cells in the brain, inflammatory infiltrates in lungs and severe degeneration and necrosis in the heart, liver and kidney [16,17]. Although these studies showed promising disease symptoms in adult mice, intranasal inoculation is an atypical route of CHIKV administration since CHIKV is not commonly known as an airborne pathogen. These investigations suggest that the age of the mice, site of virus inoculation and different genetic background can play important roles in the development of disease pathogenesis in the models.

“...the development of a humanized mouse model of Chikungunya virus infection that mimics human disease progression will be invaluable for studying host–pathogen interaction *in vivo* and for evaluating antivirals and possibly vaccines.”

Existing murine models for CHIKV suffer from the limitations of using neonatal mice or immunocompromised mouse models [11,13], since adult WT mice were mostly asymptomatic with sc. or id. virus inoculations [11,14]. The lack of appropriate models that accurately replicate human disease and physiology has no doubt contributed to the high failure rate (50%) of investigational new drugs in Phase II clinical trials [18]. The humanized mouse model largely overcomes all the drawbacks inherent to the existing models. First, the NOD/SCID/IL2rg^{-/-} background provides a platform for studies to be conducted under physiological conditions. Second, humanized mice have a full set of human immune cells, including NK cells, macrophages, dendritic cells, T cells and B cells [19]. It is expected that the development of an *in vivo* platform with an intact human immune system will be ideal for investigating the roles of immune cells in the pathogenesis of

CHIKV infection and mediating the efficacy of anti-CHIKV therapeutics.

Humanized mice have been developed by the adoptive transfer of human hematopoietic stem cells into NOD/SCID/IL2rg^{-/-} mice [19,20]. These significant improvements on the model would greatly enhance elucidation of cellular and molecular basis of host–pathogen interactions. In addition, a simple method has been devised to enhance reconstitution of NK cells and myeloid cells in reconstituted mice by expressing appropriate human cytokines via the hydrodynamic injection of cytokine-encoding plasmids [20]. This has been shown to work for NK cells, monocytes, macrophages and dendritic cells, all of which could be important for CHIKV infection or the first line of defense against CHIKV infection.

Earlier studies strongly suggested that the human immune system generates more significant responses against different virus infections than mouse immune system [20]. The successful replication of disease progression in the humanized mouse model will enable the evaluation of clinical CHIKV isolates with distinct host response profiles, disease outcomes or viral load properties. The established model will also help to identify viral factors contributing to severe

disease outcome. Building upon the capacity to manipulate human immune cells and gene expression in humanized mice, the role of specific human immune cells and cytokines in immune response against CHIKV virus can be investigated. Identification of immune cell types and cytokines that are important for antiviral response and disease pathogenesis could lead to the identification of targets for therapeutic development. Most importantly, the development of a humanized mouse model of CHIKV infection that mimics human disease progression will be invaluable for studying host–pathogen interaction *in vivo* and for evaluating antivirals and possibly vaccines.

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