Long chikungunya? An overview to immunopathology of persistent arthralgia

Persistent arthralgia decurrent from chikungunya fever
Abstract

Chikungunya fever (CF) is an arboviruses whose manifestations are extremely diverse and it has evolved with significant severity in recent years. The clinical signs triggered by Chikungunya virus (CHIKV) are similar to the manifestations of other arboviruses; in general, fever starts abruptly and reaches high levels followed by severe polyarthralgia and myalgia, as well as an erythematous or petechial maculopapular rash, which varies in severity and extent. Around 40 to 60% of affected individuals report persistent arthralgia, which can last from months to years. The symptoms of CF mainly represent the tissue tropism of the virus rather than the immunopathogenesis triggered by the host's immune system. The main mechanisms associated with arthralgia have been linked to an increase in Th17 cells and a consequent increase in receptor activator of nuclear factor kappa-B ligand (RANKL) and bone resorption. This review suggests that persistent arthralgia is the result of the permanence of viral antigens post-infection, and the constant activation of SLAM family member 7 (SLAMF7) in synovial macrophages that leads to a local infiltration of CD4+ T cells, which sustains the inflammatory process in the joints through the secretion of pro-inflammatory cytokines. The term "long chikungunya" was used in this review to refer to persistent arthralgia since, due to its manifestation over long periods after the end of the viral infection, this clinical condition seems to be characterized more as a sequel than as a symptom, since there is no active infection attached.

Key Words: Chikungunya; Immunopathology; Inflammation process; Persistent arthralgia; SLAMF7.

Core Tip: This review of Chikungunya Fever (CF) focuses on one of the most prevalent and important symptoms of the disease - arthralgia. The authors propose an approach to explain the persistence of arthralgia for a long time after the resolution of the infection, based on the sustained inflammatory response, mainly by macrophages and TH17 cells. Additionally, it is suggested that, given the context, persistent arthralgia is a sequel of CF and could therefore be termed "long Chikungunya."

INTRODUCTION

Chikungunya fever (CF) is a disease caused by the Chikungunya virus (CHIKV), an arbovirus of the Togaviridae family, genus Alphavirus, which has positive single-stranded RNA genetic material[1,2]. CF is transmitted primarily by mosquitoes of the *Aedes* genus, including *Aedes aegypti, Aedes furcifer, Aedes africanus*, and *Aedes albopictus*[2,3].

Transmission can occur through both urban and sylvatic cycles[4,5]. In the urban cycle, transmission occurs between humans and mosquito vectors, while the sylvatic cycle involves non-human primates[5,6]. In addition to transmission through mosquito bites, transmission through blood transfusions is also possible in the urban cycle[7]. The possibility of vertical transmission from an infected mother to fetus and sexual transmission has also been proposed. Sexual transmission has not yet been confirmed, and viruses have only been found in semen[4,6,8].

Since its first recorded appearance in 1952 in Tanzania, Africa, CHIKV has garnered attention for its recurrence, with outbreaks occurring every 7-20 years. In 1958, CHIKV began to be reported in central and southern regions of Africa (Uganda and the sub-Saharan region), followed by outbreaks in Asia between 1958 and 1973, and Kenya in 2004. From Kenya, it spread to the Indian Ocean, India, and Southeast Asia, resulting in more than 6 million cases. Recent outbreaks have been notable for the high number of infected individuals. In addition to the outbreak in India, Comoros recorded 215,000 cases of the disease in 2005, with a further 255,000 cases reported on Reunion Island, east of Madagascar, between 2005 and 2006[2,9]. Since 2007, health agencies have
increased their focus on CF and its causative agent due to its spread to regions of the world previously unaffected\cite{2,10}.

In addition to the significant number of cases and its ability to spread, the occurrence of fatal cases contrasts with CF's typical status as a self-limiting and mild disease. Reports of new modes of CF transmission, such as vertical transmission, have also contributed to increased awareness of the disease, which has regained attention recently due to the exponential advancement of global warming. This partly explains the presence of CHIKV in regions previously less affected, such as Europe and America\cite{8,11,12}.

In Brazil, especially following the 2014 outbreak in the Northeast region, an association was observed between CF and other arboviruses - Zika and Dengue virus. This association indicates not exacerbation in CF cases, but rather complications in dengue cases leading to hospitalizations\cite{13}.

Much of the impact caused by CHIKV and CF stems from the virus's ability to easily adapt to new locations, owing to its capacity to attract new species of anthropophilic vectors\cite{12}.

The repercussions of CF extend beyond the realm of health, affecting the economy, social welfare, and other areas. A qualitative study conducted in Curaçao revealed that the social impacts of CF varied; depending on the manifestation, duration, and severity of the disease. Patients reported social isolation, inability to engage in physical and daily activities due to physical limitations. Regarding emotional impact, there were reports of stress, anxiety, shame, frustration, despair, feelings of social exclusion, and even personality dissociation. No significant financial impacts were observed, as Curaçao has a public health system that mitigated the damage\cite{14}.

CF has an incubation period of 3 to 7 days\cite{2,3,15}. There are three clinical stages defined in CF - the acute stage from the 1st to the 21st day of infection; post-acute stage from the 21st day to the 3rd month, and chronic stage from the 3rd month onwards\cite{16}. Occasionally, infections can be asymptomatic, but these represent a minimal proportion of infected individuals\cite{2,3}. 

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Serological data can indicate leukopenia, thrombocytopenia, and elevated levels of liver transaminases\cite{17}. Some possible findings in individuals experiencing arthralgia include joint effusion, bone erosion, spinal cord edema/erosion, synovial thickening, tendonitis, and tenosynovitis, which can be detected by nuclear magnetic resonance imaging\cite{18}.

Clinical manifestations can be acute or chronic. During the acute phase, symptoms may include arthralgia, with or without fever exceeding 38.9 °C, low back pain, headache, fatigue, oligoarthralgia, or polyarthralgia (typically bilateral, predominantly affecting large and peripheral joints such as knees, ankles, wrists, shoulders, and phalanges), ocular hyperemia, oral ulcers\cite{2,3,19}, macular or maculopapular skin lesions that are swollen or pruritic, typically affecting the palms of the hands, soles of the feet, torso, and face. Gastrointestinal symptoms such as nausea, vomiting, and diarrhea may also be present during the acute phase, along with erythema, asthenia, conjunctival effusion, persistent conjunctivitis, and cervical lymphadenopathy, though the latter is less common. The severity and presence of these symptoms are associated with viral load, considered high when it ranges from 105 to 109 copies of viral RNA per milliliter\cite{8}, as well as age and biological sex\cite{2,19}.

Other atypical manifestations associated with CF have also been reported, such as Guillain-Barré syndrome, partial or total alopecia (predominantly in women), uveitis, and retinitis. Fever, loss of appetite, apnea, skin manifestations, distal and cerebral edema, encephalitis, hemorrhage, cardiac symptoms (myocarditis)\cite{2,20}, respiratory issues (Acute Respiratory Distress Syndrome - ARDS), renal complications (rhabdomyolysis, acute interstitial nephritis, thrombotic microangiopathy, and kidney damage)\cite{20}, and gastrointestinal symptoms have been reported in vertically infected neonates.

In infants, bullous lesions have been reported on the second day following the febrile state. There have also been reports of deaths due to CF in neonates, immunocompromised individuals, and the elderly, possibly linked to neurological
disorders\textsuperscript{[21]}, such as encephalitis, encephalopathy, cognitive disorders, mood swings, depression, confusion, and memory loss\textsuperscript{[8,20]}.

During the chronic phase of CF, the main symptom is persistent arthralgia, which, when it appears after the acute phase of the disease (7-10 days)\textsuperscript{[2]}, can last from weeks to years, depending on the affected population, age, and the presence of comorbidities, affecting both peripheral and large synovial joints\textsuperscript{[2,8]}. Additionally, alopecia, depression, mood swings, sleep disturbances, blurred vision, and memory loss have also been associated with the chronic phase of CF\textsuperscript{[2,3]}.

Initially, CHIKV remains present in the blood and lymph, characterizing the acute phase of CF. As the disease progresses, other organs and cell types become infected due to the hematogenous distribution route. Cell types include natural killer (NK) cells, T and B cells, dendritic cells (DC), macrophages, synovial fibroblasts, endothelial cells, and myocytes.

The chronic condition of CF can mimic arthritis, particularly rheumatoid arthritis, with symptoms such as joint effusion, bone erosions, medullary edema, synovial thickening, tendonitis, and tenosynovitis, which are present in around 55\% to 65\% of cases. Among these cases, 90\% reported bilateral joint involvement, 63\% reported joint edema, and 39\% experienced chronic myalgia. Middle-aged individuals and women are predominantly affected\textsuperscript{[23]}.

Regarding CF, studies indicate that the persistence of chronic symptoms is sustained by a prolonged inflammatory response\textsuperscript{[2,4,23,24]}. In rheumatoid arthritis, high levels of IL-12 are found, which is responsible for bone degradation and, consequently, arthralgia. Similarly, CHIKV induces the proliferation of osteoclasts by proliferating in synoviocytes\textsuperscript{[23,25]}.

Despite being a serious disease in many respects, there is still no vaccine, and pharmacological treatment is neither specific nor effective, as it currently relies on symptom management using NSAIDs (non-steroidal anti-inflammatory drugs) in conjunction with corticosteroids and antipyretics\textsuperscript{[5,7,8,26,27,28]}. 

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Patients with polyarthritis may present with other associated manifestations, characteristic of the continuation of an inflammatory process, such as swelling, and may therefore require corticosteroid therapy\textsuperscript{26}. Due to the clinical similarity between the arthralgia experienced by patients infected with CHIKV and rheumatoid arthritis, treatment typically involves the use of disease-modifying anti-rheumatic drugs (DMARDs), such as methotrexate, sulfasalazine, leflunomide, and hydroxychloroquine\textsuperscript{3, 28}. In cases where there is an inadequate response to methotrexate, satisfactory therapeutic outcomes have been reported with the administration of TNF blockers, such as etanercept\textsuperscript{28}. One challenge in developing drugs against CHIKV is the virus's intrinsic ability to mutate, potentially leading to resistance to antivirals\textsuperscript{29}. In fact, the exact immunological mechanisms involved in CF symptoms need to be better understood so that targeted management and treatment can be devised in search of more effective results.

**CHIKV INFECTION CHRONICITY**

CHIKV can be classified as an arthritogenic virus, similar to other clinically relevant alphaviruses, as it typically induces debilitating musculoskeletal diseases characterized by myalgia, arthralgia, and arthritis\textsuperscript{30}. It is estimated that 30\% to 60\% of infected individuals develop long-term sequelae, with symptoms persisting for several years\textsuperscript{3,31,32,33}. Over a third of patients report persistent or recurrent polyarthritis, with approximately half of them experiencing chronic rheumatic manifestations\textsuperscript{34,35}.

In preclinical infection trials, it was observed that CF symptoms primarily reflect the tissue tropism of the virus rather than the immunopathogenesis triggered by the host's immune system. These trials demonstrated that the severity of the infection is directly linked to the inefficiency of type I-IFN signaling\textsuperscript{36}. Another study revealed the presence of type I-IFN in the synovial tissue of patients with chronic CHIKV infection\textsuperscript{37}.

Fibroblasts are the primary targets of CHIKV, and as these cells are found in tissues such as joint capsules, fascia, and muscle insertions, they account for the
pronounced intensity of myalgia reported by patients, as these structures contain numerous nociceptive nerve endings\textsuperscript{[36]}. Additionally, there is viral tropism for blood monocytes and joint macrophages, directly contributing to the initiation of an inflammatory process during acute infection\textsuperscript{[5,37]}.

In studies conducted on non-human primates, the CHIKV genome was identified in splenic macrophages approximately 3 months post-infection, suggesting the significance of macrophages in viral persistence. Furthermore, the prolonged presence of viral antigens in lymph nodes, liver, and muscles supports the notion that macrophages act as crucial viral reservoirs\textsuperscript{[38]}. Additionally, viral tropism towards muscle satellite cells has been observed in \textit{ex vivo} and \textit{in vitro} studies\textsuperscript{[39]}.

Viral persistence has been associated with the inefficiency of the host's immune system and the effectiveness of viral evasion mechanisms\textsuperscript{[37]}. In a human study, the severity of the infection was found to be correlated with elevated serum levels of pro-inflammatory cytokines, such as IL-6 and IL-1\beta, along with a decrease in RANTES levels. During the acute phase, an inflammatory pattern predominates, driven by a Th1 immune response, with circulating cytokine levels even more pronounced in individuals with high viral loads. However, as the infection progresses to symptomatic stages, there is a shift towards Th2 response markers, such as IL-7, IL-10, and IL-15\textsuperscript{[40,41]}.

The identification of IL-7 and IL-15 in the tissue and synovial fluids of patients with rheumatoid arthritis suggests that these cytokines may be implicated in the development of arthralgia associated with CHIKV infection\textsuperscript{[40,42,43]}. IL-15 produced by synoviocyte fibroblasts induces the expression of IL-17, which has been linked to the pathogenesis of rheumatoid arthritis and the chronic phase of CHIKV infection\textsuperscript{[41,43]}. Osteoblast infection is also observed in patients with chronic arthritis, leading to detrimental effects on bone mineralization due to the inhibition of osteoprotegerin by IL-6 present in infected joints\textsuperscript{[3,5,31]}.

Persistent arthralgia typically emerges after the resolution of the acute CF phase, and despite its chronic nature, little is known about the mechanisms and factors
contributing to its progression, although various studies suggest different risk factors.

Patients with chronic arthralgia exhibit fibroblast hyperplasia, angiogenesis, tissue damage due to elevated levels of metalloproteinase-2, cell death, and infection of perivascular macrophages in synovial tissue.

Persistent arthralgia resulting from CHIKV infection also demonstrates elevated serum concentrations of IL-1Ra, IL-1β, IL-6, IL-7, IL-8, IL-12, IL-15, and IFN-α, including IL-17, a cytokine prominent in rheumatoid arthritis; this indicates that the persistent arthralgia of CF physiologically resembles rheumatoid arthritis. However, unlike rheumatoid arthritis, which exhibits serum levels of anti-CCP (anti-cyclic citrullinated peptide) and anti-RF (rheumatoid factor) antibodies, along with an increase in the CCL5/RANTES chemokine ratio, the persistent arthralgia of CF is characterized by the presence of anti-CHIKV IgM or IgG, a decrease in the CCL5/RANTES ratio, and an increase in GM-CSF (granulocyte macrophage-colony stimulating factor) and TNF-α.

An in vivo study comparing serum levels of TNF-α, IL-13, IL-2, and IL-4 during acute infection in patients who developed chronic arthralgia and those without persistent manifestations over a 20-month period post-infection observed that an intense cytokine response during the acute phase led to a reduced incidence of chronic arthralgia, whereas low cytokine levels were associated with pronounced chronic joint pain.

**ARTHRALGIA TRIGGERED BY CHIKV**

The immunopathogenic mechanisms of CF are akin to those responsible for the immune response against CHIKV. Acute CHIKV infection is characterized by elevated serum levels of pro-inflammatory chemokines, such as CCL2, CCL4, CXCL9, and CXCL10, as well as cytokines IFN type 1, IFN-γ, IL-6, IL-8, IL-17, growth factors, and GM-CSF (granulocyte macrophage-colony stimulating factor). This inflammatory microenvironment triggers a robust migration of phagocytic cells such as macrophages and activation of CD8+ T cells and NK cells to eradicate the viral agent. Although
macrophages are pivotal for the protective response, their presence in the joints contributes to the inflammatory process\cite{46}.

The number of effector lymphocytes increases during the pathogenic process of CF, and this augmentation correlates with the onset of arthralgia\cite{47}. There is a differentiation of T cells into the Th17 subtype, observed in animal models of rheumatoid arthritis and associated with pain manifestation in clinical settings\cite{23}. Thus, Th17 polarization leads to elevated circulating concentrations of IL-17, linked to bone matrix destruction and stimulation of other cytokines, pro-inflammatory chemokines, and matrix metalloproteinases (MMPs), promoting cartilage degradation. Indeed, MMP2 was found in high levels in the synovial fluid of patients with chronic arthralgia\cite{48,49}.

Therefore, IL-17 is crucial for arthralgia development, playing a role in bone resorption and weakening through RANKL (Receptor activator of nuclear factor kappa-B ligand) expression, which binds to RANK and regulates osteoclast differentiation, thereby increasing bone resorption and exacerbating joint pain\cite{50}. It is hypothesized that the increase in CD4+ T cells in the joint inflammatory microenvironment may worsen the condition due to intense TNF-\( \alpha \) release, as this cytokine contributes to the pathogenesis of psoriatic arthritis and rheumatoid arthritis\cite{51,52,53}.

Studies suggest that NK cells are also involved in acute arthralgia caused by CHIKV in murine models\cite{54,55,56}, as the increased presence of these cells in the synovial region correlates with the arthralgic mechanism in rheumatoid arthritis through the action of TNF-\( \alpha \) and IFN-\( \gamma \), although this mechanism is not fully understood\cite{57,55}. Granzyme A, released by CD8+ T cells and NK cells, also plays a significant role in CHIKV-induced arthralgia\cite{58}. Granzyme A promotes the degradation of type IV collagen and lymphocyte migration to the synovial joint\cite{59,60,61}. This association is underscored by higher serum levels of granzyme A in CHIKV-infected individuals compared to uninfected individuals, along with its proteolytic and pro-inflammatory activity\cite{53,60,62,63,64} (Figure 1).
IMMUNOPATHOLOGY OF PERSISTENT ARTHRALGIA

Some proposals attempt to explain the chronic pathogenesis of CHIKV in the joints. The establishment of the virus, through tissue tropism, in fibroblasts, satellite cells, and myoblasts\textsuperscript{[36, 39, 65]} makes these cells important reservoirs for the persistence of viral antigens, such as viral RNA, in the affected organism, even after the acute infection has resolved\textsuperscript{[37, 66, 38]}. The presence of these antigens in the joints may be one of the causes of persistent arthralgia, although the mechanisms are not fully understood.

In addition to antigenic persistence, the presence of T cells in the joints, in a chronic state, can be correlated with an increase in local IL-17 levels and other pro-inflammatory cytokines, which exacerbate joint pain\textsuperscript{[67]}. The actions of IL-17, along with RANKL, are related to the development of arthralgia, as previously mentioned\textsuperscript{[23, 68, 69, 41, 70]}.

While macrophages are essential phagocytes for controlling numerous microbial infections, they can contribute to an increase in acute inflammation, directing it towards a chronic state and causing tissue damage\textsuperscript{[71, 72]}. Dysfunctional macrophages are commonly found in autoimmune diseases such as rheumatoid arthritis\textsuperscript{[73, 74]}. Macrophage overactivation occurs through receptors and signaling molecules in the infectious microenvironment, and some studies have shown that the SLAMF7 receptor (SLAM family member 7) or CD139 plays a crucial role in transforming these phagocytic cells into an explosive, highly inflammatory, and potentially pathogenic state\textsuperscript{[75, 76]}. In an unstimulated state, the SLAMF7 receptor may be expressed in plasma cells, NK cells, B and T cells, albeit at low levels in macrophages\textsuperscript{[77, 78]}. In vitro treatment of macrophages with IFN-$\alpha$ has been shown to increase SLAMF7 expression, as well as IFN-$\alpha$ and TNF-$\alpha$\textsuperscript{[76]}. IFN-$\alpha$ has been identified as a key regulator of SLAMF7; activation of the SLAMF7 receptor by r-SLAMF7 protein led to up-regulation of TNF-$\alpha$, IL-1$\beta$, IL-6, CCL3, CCL4, CXCL1, CXCL2, and CXCL8, suggesting an intrinsic up-regulation feedback loop\textsuperscript{[76]}. Additionally, an increase in IL-6 and TNF-$\alpha$ levels was observed after stimulation\textsuperscript{[76]}.
The initial SLAMF7 activation sequence involves IFN- expression. IFN- potentiates and increases the number of SLAMF7 receptors on the surface of macrophages, and after the initial stimulus, receptor engagement triggers a highly potent activation of the inflammatory state in these cells[76]. Furthermore, after SLAMF7 induction by IFN-, TNF- from the microenvironment can recruit molecules from autocrine amplification pathways. This suggests that TNF- participates in an additional step in the maintenance of the inflammatory process[76].

During the acute viral infection phase, the host immune system responds to the infection by releasing IFN- by NK cells, antigen-presenting cells, and B cells; in addition to acting in an autocrine and paracrine manner in Th1 cell differentiation, intervening in viral replication[79, 80, 81]. IFN- release, along with macrophage/monocyte chemotaxis by the chemokines CCL2 and CCL4, may lead to increased SLAMF7 receptor expression in phagocytic cells that have migrated to the inflammatory site[46, 82, 76].

Thus, in the long term, the activation of SLAMF7 receptors in synovial macrophages associated with the persistence of viral antigens promotes the constant presence of activated CD4+ T cells in the joints; together with macrophages, T cells lead to an increase and continuous release of TNF-, which in turn acts in an autocrine manner to amplify inflammatory signaling pathways[83, 84, 47, 85, 86, 76]. This condition contributes to a state of constant inflammatory activation, perpetuating the release of cytokines that allow the configuration of an inflammatory joint microenvironment, resulting in greater tissue damage and clinical worsening (Figure 2).

CONCLUSION

The arthralgia is one of the most prevalent and relevant symptoms of CF in both the acute and chronic phases of the disease. The fact that arthralgia is reported by individuals months and even years after the infection has resolved characterizes this manifestation as persistent. Although arthralgia was triggered by the viral infection, persistent arthralgia is not supported by the presence of the infection. In this context,
persistent arthralgia, in the absence of infection, classifies this clinical condition as a sequel rather than a symptom. Therefore, in comparison to similar cases involving viral agents, this post-infection condition could be determined, for the first time, as "long chikungunya".