

# Chikungunya Fever: Focus on Peripheral Markers of Pathogenesis

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(See the article by Angela Chow et al, on pages 149–157.)

Chikungunya fever, a previously neglected arboviral disease, is caused by mosquito-transmitted Chikungunya virus (CHIKV). Attention of scientists and the media has focused on CHIKV since its reemergence in 2005 on La Reunion island, a French island of the Indian Ocean [1]. Since then, CHIKV has caused several epidemics [2, 3], with millions of cases mainly centered around the Indian Ocean. An outbreak also occurred in Italy, and thousands of cases have been diagnosed in other countries in travelers [2, 3].

The changing disease pattern, and adaptation of CHIKV from *Aedes aegypti* to another mosquito vector, *Ae. albopictus*, are important new features that impact public health [3–5]. Indeed, the spread of *Ae. albopictus* in temperate countries introduces a new risk of epidemics in countries where the entire population is immunologically naive to this infection [6]. Interestingly, the magnitude of the recent outbreaks can be related to CHIKV genome microevolution and adaptation to *Ae. albopictus* [7, 8]. However, questions remain about the role of

such microevolution on viral virulence and severity of the associated disease.

The symptoms and signs of CHIKV-induced disease include rash, fever, arthralgia, and myalgia [9, 10]. Until 2006, the disease was considered benign, and very little was known regarding the prevalence of each clinical manifestation among infected persons. The duration of the associated arthralgia was particularly unclear, though it was known that durations of months occurred but were rare. Since the epidemic on La Reunion Island, knowledge concerning the clinical presentation of the disease has dramatically increased. At present, “classical” and “new” clinical patterns of the disease are recognized. During the Reunion epidemic, but also in India thereafter, neurological forms of the disease [11, 12] and life-threatening cases were reported, particularly in infants and the elderly. Since then, the prevalence of life-threatening disease has been recognized to be quite low (below 1%), but as infection rates might be >30% for a naive population, such rare but severe cases nevertheless represent an important topic for research [13]. The Reunion Island outbreak appeared to involve more severe and persistent rheumatic disease cases, with several studies showing that 50%–75% of CHIKV-infected adults still suffered from joint pain 1 year after infection [14], although the percentage is different

in other locations [10]. Today we neither know what determines persistent clinical disease nor if this is related to acute disease severity.

In a first approach to answer these questions, research teams tried to define target cells and CHIKV replication patterns in tissues. Mouse and nonhuman primate animal models were developed and CHIKV persistence in tissues and parameters associated with arthralgia were studied, together with scarce data obtained from human biopsies [1, 15–17]. The severity of the disease in the acute phase was related to the viral load, but no clear relationship with chronic arthralgia was evident. Persistent arthralgia is at best subjective in humans and is difficult or impossible to assess in animal models. It is thus very important to undertake careful studies in human patient cohorts to determine the factors that may play a role in long-term persistent arthralgia.

In an extension of their previous work [18], Chow et al [19] in this issue of the *Journal* present a case-control longitudinal study including 30 patients segregated into 2 groups according to high or low viral load at the onset of the disease. This classification is easier to use than their previous one that took into account the severity of the acute phase. Of note, the groups obtained by the 2 methods are consistent. They performed a longitudinal follow-up of these patients at 4 time points designated

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“acute” (median, 4 days), “early convalescence” (median, 10 days), “late convalescence” (4–6 weeks) and “chronic” (2–3 months) phases. Four patients had chronic arthralgia, allowing an interesting, although very preliminary, assessment of the markers that may associate with this chronic pain. This is the first longitudinal study involving so many patients, as previous work has mainly described cross-sectional analyses [20–22] and focused on viral load, antibody response, or clinical description of the acute phase [23].

This article by Chow et al [19] clearly adds a step toward the understanding of this disease. It confirms high levels of pro-inflammatory cytokines in the acute phase [15, 16]. Different cytokine modulation patterns are described according to their kinetics, and numerous cytokines remain elevated during the chronic phase when most patients have recovered. Although only 4 subjects presented persistent arthralgia, Chow and colleagues found an association with high levels of interleukin (IL) 6 and granulocyte macrophage colony-stimulating factor (GM-CSF), but not of tumor necrosis factor (TNF)  $\alpha$  and IL-1 $\beta$ , suggesting an active immunopathogenic mechanism. High levels of proinflammatory cytokines, in particular IL-6, TNF $\alpha$ , and IL-1 $\beta$ , are actively involved in other arthritides and are elevated in the serum and synovial fluid (for review, see Schaible et al [24]). Both IL-6 and TNF $\alpha$  may directly participate in pain production by their action on nociceptors in the joint and on dorsal root ganglion neurons in the spinal cord [25]. These neurons express CD130/gp130 to which a complex of IL-6 and the soluble IL-6 receptor can bind. The absence of increased levels of TNF $\alpha$  and IL-1 $\beta$  has only been assessed in the blood, a major limitation of this article. Local expression in the joint should be studied before the participation of these, and perhaps other, cytokines in the pathogenesis can be fully explored or ruled out. GM-CSF is one of the numerous proinflammatory

cytokines involved in arthritis [25, 26]. To our knowledge, there is no known direct link between this cytokine and pain. The high concentrations of GM-CSF in patients with persistent arthralgia may be a marker of persistent inflammation, or even of persistent virus infection.

Interestingly, the 4 patients with persistent arthralgia had normal levels of hepatocyte growth factor (HGF) and Eotaxin, whereas the recovered patients had elevated levels of these 2 cytokines, even though clinical recovery was total. This indicates that although there is full clinical recovery in most patients at 2–3 months after illness onset, active disease-associated mechanisms are still ongoing. HGF and Eotaxin are 2 of the “early convalescent phase” induced factors and probably participate in resolution of the inflammation and repair. HGF regulates the IL-6/IL-10 balance in favor of IL-10, at least in the mouse model of endotoxemia [27], and it is tempting to speculate that the increased IL-6 levels in Chikungunya arthralgia are secondary to a defect in HGF expression. Eotaxin is a Th2 chemokine and a natural antagonist of CCR2, the receptor for CCL-2/MCP-1 [28]. CCL-2 is a major chemoattractant for monocytes/macrophages in tissues and is highly expressed during the acute phase of the infection [29–31]. Thereafter, CCL-2 levels decline, but at 2–3 months they nevertheless remain higher than normal. The high level of Eotaxin in recovered patients may indicate that full clinical recovery needs active inhibition of CCR-2 signaling.

These data strongly suggest that during the chronic phase of illness, active immune mechanisms are still underway that allow clinical recovery in a majority of patients, while a minority who do not maintain such mechanisms experience persistent pain and chronic joint inflammation. Whatever the outcome, the question remains: what induces such long-lasting immune activation? Animal models may help because they allow study of virus persistence and replication

in joint and other tissues and can temporally relate this to immunological and inflammatory events. In these models, virus is mainly associated with cells of the monocyte-macrophage lineage, but fibroblasts and dendritic cells may also be involved [16,17].

Interestingly, the results obtained by Chow et al [19] differ from those reported by Hoarau et al [15] concerning 9 patients with persistent arthralgia and 6 who had fully recovered. First, all 4 Singapore patients with persistent arthralgia had low viral load, and most had mild acute disease (1 had severe acute disease). In contrast, chronic arthralgia was associated with severe acute disease in La Réunion. The La Réunion study found persistent IFN $\alpha$  and IL-12 expression in chronic cases, whereas Chow et al [19] did not. They also found no differences in IL-6 concentration in chronic versus recovered cases. Such differences may arise from the small numbers of subjects in both studies, differences in virus strains, or differences in Chikungunya disease profiles in these 2 different populations. Further comparative studies, funded by the European Union, are ongoing to investigate the pathogenesis of the chronic phase and other aspects of Chikungunya fever.

In conclusion, the article by Chow et al [19] in this issue presents a well-conducted study that will be of particular interest in relation to future control of Chikungunya disease, a disease that seems to be endemic in Asia, and to have more severe features than previously acknowledged. Arthritogenic virus infection is a new emerging health problem in places where people already face other mosquito-borne epidemics (eg, Dengue fever). These new emerging diseases deserve studies such as that by Chow et al [19] that compare the biological features and specific immune markers of infection, and which might form the basis for future preclinical and clinical studies in both animals and humans.

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