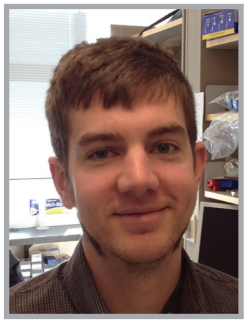
Future VIROLOGY

EDITORIAL

Mechanisms underlying the pathogenesis of arthritogenic alphaviruses: host immune responses and virus persistence



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"A better understanding of the molecular mechanisms underlying the pathogenesis of these infections will inform vaccine, antiviral and immunotherapeutic strategies to prevent and reduce morbidity in those infected."

KEYWORDS
• alphavirus • chikungunya • viral pathogenesis

Chikungunya virus (CHIKV), and several related alphaviruses, are mosquito-transmitted viruses that cause debilitating musculoskeletal disease in humans. Since 2004, CHIKV has caused millions of disease cases in the Indian Ocean region and has emerged in new areas, including Italy, France, the Middle East and the Pacific region [1]. The mosquito vectors for this virus are globally distributed in tropical and temperate zones, suggesting that CHIKV will continue to emerge in new areas. In fact, in December 2013 an outbreak of CHIKV occurred on St Martin, which has subsequently spread to multiple islands in the Caribbean, French Guiana and possibly to Mexico. This event has raised concerns of looming epidemics throughout the Americas.

Chikungunya, which translates as "that which bends up the joints", is characterized by an abrupt onset of fever and severe joint pain that may persist for weeks to years [2]. The arthralgia is typically symmetrical and affects peripheral joints, including wrists, knees, ankles and the small joints of the hand. Additional disease signs and symptoms include arthritis, with joints often exhibiting tenderness and swelling, tenosynovitis, skin rash and myalgia. In addition to these clinical features, severe neurologic and cardiac manifestations have been associated with CHIKV infection. In addition, mother-to-child transmission with high rates of morbidity have been reported [3]. Chronic CHIKV disease can be highly debilitating and have severe economic consequences [4,5], highlighting the significant public health threat posed by CHIKV and the need for continued research into the pathogenesis of these infections.

Do host immune responses contribute to acute &/or chronic CHIKV disease?

Studies from humans and animal models have shown that disease signs and symptoms following infection with arthritogenic alphaviruses are associated with the infiltration of musculoskeletal tissues with monocytes/macrophages, NK cells and T cells, as well as with elevated levels of specific cytokines and components of the

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complement cascade. In addition, MRI findings from CHIKV-infected patients showed joint effusion, bone marrow edema, synovial thickening, tendinitis and tenosynovitis [6]. Together, these data suggest an immunopathologic component to alphavirus rheumatological disease; however, the precise immunopathologic mechanisms have not yet been well defined.

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During acute CHIKV infection, a limited number of studies have shown that activated T cells and a unique subset of cytotoxic NK cells are detectable in the periphery [7,8]. Even less understood is the activation status and effector functions of musculoskeletal tissue-infiltrating immune cells. The cellular infiltrate in the synovial fluid of patients with Ross River virus (RRV) disease is predominantly monocytic, with macrophages showing an inflammatory phenotype [9]. Similarly, the synovial fluid of a patient with chronic CHIKV disease showed monocytes/macrophages and NK cells [10]. Macrophages and T cells are also detected in synovial and muscle tissue biopsies from patients suffering from chronic CHIKV disease [10,11]. The roles of these cell types, and other cell subsets, in pathogenesis remain to be elucidated and are a focus of intense research in animal models. For example, treatment of mice with clodronate-loaded liposomes, which deplete macrophages, reduced the severity of acute disease signs in CHIKV- and RRV-infected mice [12,13], suggesting a pathogenic role for macrophages during these infections. Additional studies showed that the injury to musculoskeletal tissues in RRV- or CHIKV-infected mice promotes a wound-healing response characterized by the abundance of arginase 1-expressing macrophages [14]. Mice deleted for arginase 1 in myeloid cells, which function in wound repair and immune suppression [15], had reduced viral loads and less severe tissue pathology at late times post-RRV infection [14]. These findings further implicate macrophages and specific macrophage effector functions in pathogenesis. Studies in animal models have also implicated lymphocytes in the pathogenesis of alphavirus rheumatological disease. Both *Rag1*$^{-/-}$ mice, which lack mature T and B cells, and *CD4*$^{-/-}$ mice, which lack CD4^{+} T cells, had reduced joint swelling and less severe musculoskeletal tissue injury during the acute stage of CHIKV disease [16,17]. By contrast, *Rag1*$^{-/-}$ mice had more severe musculoskeletal tissue pathology during chronic CHIKV infection [17]. These results are consistent with findings in rhesus macaques in which persistence of CHIKV correlated with defects in adaptive immunity [18], and in humans in which the appearance of neutralizing IgG3 antibodies correlated with protection from chronic CHIKV disease [19]. Together, these findings suggest both pathogenic and protective roles for immune cells in alphavirus rheumatological disease.

The host complement pathway has also been implicated in the pathogenesis of these viral infections. Activated complement was detected in the synovial fluid of RRV-infected patients [20], and the levels of MBL, an activator of the complement cascade, in the serum and synovial fluid correlated with disease severity [21]. Complement was shown to play a pathologic role in RRV-induced disease in mice, with both *C3*$^{-/-}$ and *MBL*$^{-/-}$ mice developing less severe musculoskeletal tissue damage and disease signs [20,21]. Although the role of the complement pathway in CHIKV-induced disease has not been extensively investigated, recent gene-expression analyses in mice suggest that complement is activated during CHIKV infection [22]. Thus, interfering with the complement cascade may represent a useful route for therapeutic intervention.

Another of area of intense research is the role of cytokines in alphavirus rheumatological disease, particularly due to the success of drugs targeting cytokines in the treatment of other arthritides [23]. Several studies have reported upregulation of IFN-α, IFN-γ, IL-6, MIF, CCL2 (MCP-1), CXCL9 (MIG) and CXCL10 (IP-10) during the acute stage, with levels of IL-6, IL-1β, RANTES, MCP-1, MIG and IP-10 linked to CHIKV disease severity [24–26]. Cytokines may also contribute to chronic CHIKV disease, as persistent arthralgia has been associated with elevated levels of IL-6, GM-CSF, MIG and MCP-1 [27,28]. Consistent with these data, the serum levels of MCP-1, MIG and IP-10 correlated with the degree of joint swelling in CHIKV-infected mice [29]. Furthermore, treatment of mice with bindarit, an inhibitor of MCP-1 synthesis, reduced the severity of CHIKV- and RRV-induced musculoskeletal tissue inflammation and injury [30,31], suggesting that MCP-1-mediated recruitment of macrophages contributes to disease severity. However, studies in animal models that tested the role of specific cytokines have been less clear. For example, while one study reported that genetic deletion of *IFN-γ* reduced CHIKV-induced joint swelling in mice [22], this was not observed in a second study [16]. In addition, treatment of RRV-infected mice with etanercept, an anti-TNF drug,

resulted in more severe disease [32]. Thus, further studies investigating the role for specific cytokines in the pathogenesis of alphavirus rheumatological disease are needed.

Is chronic alphavirus rheumatological disease due to chronic viral infection?

As discussed above, the development of chronic joint pain in a subset of individuals is a central feature of infection with arthritogenic alphaviruses. The cause of this persistent joint disease is unclear; however, there is little evidence for the development of autoimmunity in individuals experiencing chronic disease [2]. Thus, an unresolved question in the field is whether chronic alphavirus rheumatological disease is associated with or caused by persistent alphavirus infection.

Although data from human studies are limited, evidence that is suggestive of persistent virus infection in patients suffering from chronic alphavirus rheumatological disease has been reported. CHIKV antigen and RNA was detected in synovial tissue biopsies collected from a patient suffering from chronic joint pain, with CHIKV antigen detected in perivascular macrophages [10]. In addition, CHIKV antigen was detected in muscle satellite cells in a muscle tissue biopsy collected from of a patient during a relapse of chronic musculoskeletal pain [11]. Finally, RRV RNA was detected in knee biopsies collected 5 weeks after the onset of joint symptoms [33].

Studies in several animal models further indicate that arthritogenic alphaviruses establish chronic infections in musculoskeletal and other tissues. CHIKV RNA and antigens were detected up to 90 days postinoculation in the spleen, lymph nodes, liver and, to a lesser extent, in synovial and muscle tissue of infected cynomolgus macaques [34]. Similarly, CHIKV RNA was found to persist in the spleen of experimentally infected aged rhesus macaques [18]. In mice, infection with a recombinant CHIKV expressing luciferase resulted in detection of luciferase activity 60 days postinfection in joint-associated tissues near the site of inoculation [16], while work in our laboratory showed that CHIKV RNA persisted specifically in numerous joints, but not a variety of other tissues, for at least 16 weeks. Furthermore, the persistence of CHIKV RNA in joints was associated with chronic synovitis [17]. Although these data are not conclusive, cumulatively, they support the intriguing hypothesis that chronic alphavirus infection of joints, and perhaps other tissues, drives chronic rheumatological disease. This hypothesis raises many important questions to be addressed in future research. For example, what are the mechanisms that allow the virus to evade innate and adaptive immunity and establish persistence? What are the viral and host determinants that govern the tissue and cellular tropism during persistence? What is the virus replication strategy during persistence?

In the past decade, CHIKV and related arthritogenic alphaviruses have caused millions of cases of acute and chronic rheumatological disease. The continued re-emergence of CHIKV into new geographic areas with large naive populations puts millions more at risk and presents a significant public health concern. A better understanding of the molecular mechanisms underlying the pathogenesis of these infections will inform vaccine, antiviral and immunotherapeutic strategies to prevent and reduce morbidity in those infected.

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References

1 Tsetsarkin KA, Chen R, Sherman MB, Weaver SC. Chikungunya virus: evolution and genetic determinants of emergence. *Curr. Opin. Virol.* 1(4), 310–317 (2011).

2 Suhrbier A, Jaffar-Bandjee MC, Gasque P. Arthritogenic alphaviruses – an overview. *Nat. Rev. Rheumatol.* 8(7), 420–429 (2012).

3 Gerardin P, Barau G, Michault A *et al.* Multidisciplinary prospective study of mother-to-child Chikungunya virus infections on the island of La Reunion. *PLoS Med.* 5(3), e60 (2008).

4 Schilte C, Staikovsky F, Couderc T *et al.* Chikungunya virus-associated long-term arthralgia: a 36-month prospective longitudinal study. *PLoS Negl. Trop. Dis.* 7(3), e2137 (2013).

5 Soumahoro MK, Boelle PY, Gauzere BA *et al.* The Chikungunya epidemic on La Reunion Island in 2005–2006: a cost-of-illness study. *PLoS Negl. Trop. Dis.* 5(6), e1197 (2011).

6 Manimunda SP, Vijayachari P, Uppoor R *et al.* Clinical progression of Chikungunya fever during acute and chronic arthritic stages and the changes in joint morphology as revealed by imaging. *Trans. R Soc. Trop. Med. Hyg.* 104(6), 392–399 (2010).

7 Petitdemange C, Becquart P, Wauquier N *et al.* Unconventional repertoire profile is imprinted during acute chikungunya infection for natural killer cells polarization toward cytotoxicity. *PLoS Pathog.* 7(9), e1002268 (2011).

8 Wauquier N, Becquart P, Nkoghe D, Padilla C, Ndjoyi-Mbiguino A, Leroy EM. The acute phase of Chikungunya virus infection in humans is associated with strong innate immunity and T CD8 cell activation. *J. Infect. Dis.* 204(1), 115–123 (2011).

9 Fraser JR, Cunningham AL, Clarris BJ, Aaskov JG, Leach R. Cytology of synovial effusions in epidemic polyarthritis. *Aust. NZ J. Med.* 11(2), 168–173 (1981).

10 Hoarau JJ, Jaffar Bandjee MC, Krejbich Trotot P *et al.* Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. *J. Immunol.* 184(10), 5914–5927 (2010).

11 Ozden S, Huerre M, Riviere JP *et al.* Human muscle satellite cells as targets of Chikungunya virus infection. *PLoS ONE* 2(6), e527 (2007).

12 Gardner J, Anraku I, Le TT *et al.* Chikungunya virus arthritis in adult wild-type mice. *J. Virol.* 84(16), 8021–8032 (2010).

13 Lidbury BA, Rulli NE, Suhrbier A *et al.* Macrophage-derived proinflammatory factors contribute to the development of arthritis and myositis after infection with an arthrogenic alphavirus. *J. Infect. Dis.* 197(11), 1585–1593 (2008).

14 Stoermer KA, Burrack A, Oko L *et al.* Genetic ablation of arginase 1 in macrophages and neutrophils enhances clearance of an arthritogenic alphavirus. *J. Immunol.* 189(8), 4047–4059 (2012).

15 Munder M. Arginase: an emerging key player in the mammalian immune system. *Br. J. Pharmacol.* 158(3), 638–651 (2009).

16 Teo Th, Lum FM, Claser C *et al.* A pathogenic role for $CD4^+$ T cells during Chikungunya virus infection in mice. *J. Immunol.* 190(1), 259–269 (2013).

17 Hawman DW, Stoermer KA, Montgomery SA *et al.* Chronic joint disease caused by persistent Chikungunya virus infection is controlled by the adaptive immune response. *J. Virol.* 87(24), 13878–13888 (2013).

18 Messaoudi I, Vomaske J, Totonchy T *et al.* Chikungunya virus infection results in higher and persistent viral replication in aged rhesus macaques due to defects in anti-viral immunity. *PLoS Negl. Trop. Dis.* 7(7), e2343 (2013).

19 Kam YW, Simarmata D, Chow A *et al.* Early appearance of neutralizing immunoglobulin G3 antibodies is associated with Chikungunya virus clearance and long-term clinical protection. *J. Infect. Dis.* 205(7), 1147–1154 (2012).

20 Morrison TE, Fraser RJ, Smith PN, Mahalingam S, Heise MT. Complement contributes to inflammatory tissue destruction in a mouse model of Ross River virus-induced disease. *J. Virol.* 81(10), 5132–5143 (2007).

21 Gunn BM, Morrison TE, Whitmore AC *et al.* Mannose binding lectin is required for alphavirus-induced arthritis/myositis. *PLoS Pathog.* 8(3), e1002586 (2012).

22 Nakaya HI, Gardner J, Poo YS, Major L, Pulendran B, Suhrbier A. Gene profiling of Chikungunya virus arthritis in a mouse model reveals significant overlap with rheumatoid arthritis. *Arthritis Rheum.* 64(11), 3553–3563 (2012).

23 Choy EH, Kavanaugh AF, Jones SA. The problem of choice: current biologic agents and future prospects in RA. *Nat. Rev. Rheumatol.* 9(3), 154–163 (2013).

24 Lohachanakul J, Phuklia W, Thannagith M, Thonsakulprasert T, Ubol S. High concentrations of circulating interleukin-6 and monocyte chemotactic protein-1 with low concentrations of interleukin-8 were associated with severe Chikungunya fever during the 2009–2010 outbreak in Thailand. *Microbiol. Immunol.* 56(2), 134–138 (2012).

25 Ng LF, Chow A, Sun YJ *et al.* IL-1beta, IL-6, and RANTES as biomarkers of Chikungunya severity. *PLoS ONE* 4(1), e4261 (2009).

26 Kelvin AA, Banner D, Silvi G *et al.* Inflammatory cytokine expression is associated with Chikungunya virus resolution and symptom severity. *PLoS Negl. Trop. Dis.* 5(8), e1279 (2011).

27 Chow A, Her Z, Ong EK *et al.* Persistent arthralgia induced by Chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor. *J. Infect. Dis.* 203(2), 149–157 (2011).

28 Reddy V, Mani RS, Desai A, Ravi V. Correlation of plasma viral loads and presence of Chikungunya IgM antibodies with cytokine/chemokine levels during acute Chikungunya virus infection. *J. Med. Virol.* doi:10.1002/jmv.23875 (2014) (Epub ahead of print).

29 Gardner CL, Hritz J, Sun C *et al.* Deliberate attenuation of Chikungunya virus by adaptation to heparan sulfate-dependent infectivity: a model for rational arboviral vaccine design. *PLoS Negl. Trop. Dis.* 8(2), e2719 (2014).

30 Rulli NE, Guglielmotti A, Mangano G *et al.* Amelioration of alphavirus-induced arthritis and myositis in a mouse model by treatment with bindarit, an inhibitor of monocyte chemotactic proteins. *Arthritis Rheum.* 60(8), 2513–2523 (2009).

31 Rulli NE, Rolph MS, Srikiatkhachorn A, Anantapreecha S, Guglielmotti A, Mahalingam S. Protection from arthritis and myositis in a mouse model of acute Chikungunya virus disease by bindarit, an inhibitor of monocyte chemotactic protein-1 synthesis. *J. Infect. Dis.* 204(7), 1026–1030 (2011).

32 Zaid A, Rulli NE, Rolph MS, Suhrbier A, Mahalingam S. Disease exacerbation by etanercept in a mouse model of alphaviral arthritis and myositis. *Arthritis Rheum.* 63(2), 488–491 (2011).

33 Soden M, Vasudevan H, Roberts B *et al.* Detection of viral ribonucleic acid and histologic analysis of inflamed synovium in Ross River virus infection. *Arthritis Rheum.* 43(2), 365–369 (2000).

34 Labadie K, Larcher T, Joubert C *et al.* Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *J. Clin. Invest.* 120(3), 894–906 (2010).